# Toxicological profile for

## Lemon oils

***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*

Not applicable.

### *1.2. Synonyms*

Citrus limon oil; Lemon oil; Lemon tincture; Citrus medica var. limonum oil; Cedro oil; Citrus limon extract; Extract of lemon; Lemon extract; Lemon petitgrain oil; Lemon petigrain oil; FEMA No. 2853; HSDB 1944; Lemon oil concentrate; Oil of lemon; Oils, citrus limonia; Oils, lemon; Petitgrain lemon oil (Citrus limon (L.) Burm. f.); Zitronen oel [German]; UNII-ET5GD00TRP; UNII-I9GRO824LL [descriptors such as Guinea, Italian and distilled may be used] (ChemIDplus); Citrus limon (lemon) peel oil (CIR, 2014)

### *1.3. Molecular formula*

Unspecified (ChemIDplus)

### *1.4. Structural Formula*

Not applicable.

### *1.5. Molecular weight (g/mol)*

Not applicable.

### *1.6. CAS registration number*

8008-56-8

### *1.7. Properties*

#### *1.7.1. Melting point*

(°C): <25 (EPISuite, 2017); -40.76 (estimated) (EPISuite, 2017)

#### *1.7.2. Boiling point*

(°C): 178 (EPISuite, 2017)

#### *1.7.3. Solubility*

7.57 mg/L or 13.8 mg/L at 25°C (EPISuite, 2017)

#### *1.7.4. pKa*

No data available to us at this time.

#### *1.7.5. Flashpoint*

(°C): 46 (closed cup) (NZ EPA CCID)

#### *1.7.6. Flammability limits (vol/vol%)*

No data available to us at this time.

*1.7.7. (Auto)ignition temperature*

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

*1.7.8. Decomposition temperature*

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

*1.7.9. Stability*

No data available to us at this time.

*1.7.10. Vapor pressure*

1.44 mm Hg at 25°C (EPISuite, 2017)

*1.7.11. log Kow*

4.38 or 4.57 (EPISuite, 2017)

## *2. General information*

### *2.1. Exposure*

Cosmetics: Yes (Cosmetics Bench Ref, 1993).

Food: Yes (Burdock, 2010).

Environment: No evidence.

Pharmaceuticals: Yes (Martindale, 1993).

Lemon oil is found in a variety of foods. Burdock (2010) lists an individual's daily consumption as being 3.6633 mg/kg bw/day. The FEMA PADI (possible average daily intake) is 53.958 mg.

Reported uses as a flavouring in:

| Food category | Usual (ppm) | Max (ppm) | Food category | Usual (ppm) | Max (ppm) |
|---|---|---|---|---|---|
| Alcoholic beverages | 122.8 | 173.8 | Gravies | 41.37 | 106.8 |
| Baked goods | 212.8 | 412.9 | Hard candy | 431.4 | 2017 |
| Breakfast cereals | 200 | 400 | Jam, jellies | 125 | 125 |
| Chewing gum | 376.1 | 1692 | Meat products | 18.68 | 20.02 |
| Condiments, relishes | 151.3 | 306.5 | Nonalcoholic beverages | 50.7 | 108.7 |
| Fats, oils | 20.0 | 20.0 | Soft candy | 232 | 456 |
| Frozen dairy | 126.1 | 254.4 | Soups | 5.53 | 7.0 |
| Gelatins, puddings | 108.5 | 265.2 | | | |

As taken from Burdock, 2010.

Lemon oil is used as a fragrance in perfumed cosmetics and is typically found at up to 0.4% in detergents, creams and lotions (CoE, 2001).

Used as an astringent and tonic ingredient (Citrus limon fruit oil; CAS RN 8008-56-8), a fragrance, perfuming and skin conditioning ingredient (Citrus limon peel oil; CAS RN 8008-56-8/84929-31-7), a humectant and skin conditioning ingredient (Citrus limon bud extract and Citrus limon flower

water; no CAS RNs given), a skin conditioning - emollient and skin conditioning agent (Citrus limon flower extract; no CAS RN given), an antimicrobial, hair conditioning, skin conditioning and skin protecting ingredient (Citrus limon callus culture extract; no CAS RN given), an emulsion stabilising ingredient (Citrus limon fiber; no CAS RN given), hair and skin conditioning ingredient (Hydrolyzed lemon fruit extract; no CAS RN given) in cosmetics in the EU.

As taken from CosIng (Cosmetic substances and ingredients database). Available at https://ec.europa.eu/growth/tools-databases/cosing/, undated.

Lemon (steam distilled) oil; lemon oil folded (5X); lemon oil, expressed; lemon essence oil; lemon oil, distilled; lemon oil, washed and lemon oil folded (10X) (all CAS RN 8008-56-8) are listed as fragrance ingredients by IFRA and oil of lemon (CAS RN 8008-56-8) by the US EPA InertFinder Database (2022).

Lemon oil (CAS RN 8008-56-8) is listed as an ingredient (at given concentrations, where specified) in auto, home maintenance (0-5%), inside the home (<0.1-<1%), “old” landscaping/yard, personal care (0.1-1%) and pet care products by the CPID.

“Citrus limon (lemon) peel oil has the most reported uses in cosmetics and personal care products, with a total of 490; more than half of the uses are in leave-on skin care preparations.”

“In a use concentration survey…., citrus limon (lemon) peel oil had a maximum use concentration range of 0.0001% to 0.5%, with 0.5% reported in “other” skin care preparations.”

As taken from CIR, 2014.

“Uses:

FLAVORING MEDICAMENTS, LIQUEURS, PASTRY, FOODS, BEVERAGES; IN PERFUMES [The Merck Index. 9th ed. Rahway, New Jersey: Merck & Co., Inc., 1976., p. 883] **PEER REVIEWED**”

As taken from HSDB, 2002

According to Health Canada’s Natural Health Products Ingredients Database, the following substances are used in non-medicinal natural health products for the indicated purposes:

Citrus limon (lemon) fruit extract (no CAS RN given) is used as a skin-conditioning agent for topical use;

Citrus limon (lemon) peel extract (no CAS RN given) is used as a skin-conditioning/emollient agent for topical use;

Citrus limon (lemon) peel oil (no CAS RN given) is used as a masking and skin-conditioning agent. It is also used as a fragrance ingredient for topical use at “up to 0.5%, when formulated to be non-sensitizing and non-irritating, and provided that leave-on products do not contain more than 15 ppm 5-methoxypsoralen”;

Lemon essential oil (no CAS RN given) is used as a preservative antioxidant (at up to 0.1%) for oral use and a flavour enhancer for oral use;

Lemon essential oil distilled (no CAS RN given) is used as a flavour enhancer for oral use;

Citrus medica limonum (lemon) fruit extract (no CAS RN given) is used as a flavour enhancer, fragrance ingredient, skin-conditioning and skin-conditioning (occlusive) agent.

As taken from Health Canada, 2022.

### *2.2. Combustion products*

No data available to us at this time.

*2.3. Ingredient(s) from which it originates*

Lemon oil is prepared by mechanical treatment of fresh peels without heat (CoE, 2001).

"...from fresh peel of citrus limonum (l) risso (C medica var limon l), rutaceae. constituent 90% of limonene, terpinene, phellandrene, & pinene combined; 4-6% aldehydes calculated as citral, some citronellal, geranyl acetate, sesquiterpenes. [The Merck Index. 9th ed. Rahway, New Jersey: Merck & Co., Inc., 1976., p. 883] **PEER REVIEWED**"

As taken from HSDB, 2002

"Citrus Limon (Lemon) Fruit Oil Citrus Limon (Lemon) Fruit Oil (no CAS RN specified) is the volatile oil obtained from the fruit of Citrus limon."

As taken from CIR, 2017.

| | | |
|---|---|---|
| CITRUS LIMON FRUIT OIL | 8008-56-8 | Citrus Limon Fruit Oil is the volatile oil obtained from the fruit of Lemon, Citrus limon (L.), Rutaceae |
| CITRUS LIMON PEEL OIL | 8008-56-8 / 84929-31-7 | Citrus Limon Peel Oil is the volatile oil obtained from the fresh peel of the Lemon, Citrus limon (L.), Rutaceae |
| CITRUS LIMON BUD EXTRACT | No CAS RN listed | Citrus Limon (Lemon) Bud Extract is the extract of the buds of Citrus limon, Rutaceae. |
| CITRUS LIMON FLOWER EXTRACT | No CAS RN listed | Citrus Limon (Lemon) Flower Extract is the extract of the flowers of Citrus limon, Rutaceae. |
| CITRUS LIMON FLOWER WATER | No CAS RN listed | Citrus Limon (Lemon) Flower Water is an aqueous solution of the steam distillates obtained from the flowers of Citrus limon, Rutaceae. |
| CITRUS LIMON CALLUS CULTURE EXTRACT | No CAS RN listed | Citrus Limon Callus Culture Extract is the extract of a culture of the callus of Citrus limon, Rutaceae. |
| CITRUS LIMON FIBER | No CAS RN listed | Citrus Limon Fiber is the fiber obtained from the pulp of Citrus limon, Rutaceae (The accepted scientific name for Citrus limon is Citrus x limon). |
| HYDROLYZED LEMON FRUIT EXTRACT | No CAS RN listed | Hydrolyzed Lemon Fruit Extract is the hydrolysate of Citrus Limon, Rutaceae, Fruit Extract derived by acid, enzyme or other method of hydrolysis. |

As taken from CosIng (Cosmetic substances and ingredients database). Available at https://ec.europa.eu/growth/tools-databases/cosing/, indated.

## *3. Status in legislation and other official guidance*

"FDA Requirements:

LEMON: GRAS (II); LEMON PEEL: GRAS (II); PETITGRAIN LEMON: GRAS (II).

[Fenaroli's Handbook of Flavor Ingredients. Volume 1. Edited, translated, and revised by T.E. Furia and N. Bellanca. 2nd ed. Cleveland: The Chemical Rubber Co., 1975., p. 390] **PEER REVIEWED**"

As taken from HSDB, 2002

In the US, lemon, oil (Citrus limon (L.) Burm. F.; CAS RN 8008-56-8) is listed in the FDA's Substances Added to Food (formerly EAFUS) database as a flavoring agent or adjuvant and is included on a list of essential oils, oleoresins (solvent-free) and natural extractives (including distillates) that are considered generally recognised as safe (GRAS) for their intended use (21 CFR 182.20). It is also included under 21 CFR sections 146.114 (Lemon juice), 146.120 (Frozen concentrate for lemonade), 161.190 (Canned tuna), 172.230 (Microcapsules for flavoring substances) and 173.240 (Isopropyl alcohol) (FDA, 2022a,b).

d-Limonene is the major constituent (80-90%) of lemon oil (bibra, 1998).

An ADI “not specified” was recommended by JECFA at its 41st meeting since it was believed that the total daily intake (from its use in food and background levels in food) would not represent a hazard to health (JECFA, 1993a).

Codex Alim.: Not listed

C of E no.: 139a

FEMA no.: 2853

TLV/OEL: Not listed

Cosmetics (UK): Not listed in Schedule 1

Oil of lemon (CAS RN 8008-56-8) is listed in the US EPA InertFinder Database (2022) as approved for non-food and fragrance use pesticide products.

According to SCCP (2005), lemon oil “may be used in cosmetic products provided that the total concentration of furocoumarin-like substances in the finished cosmetic product does not exceed 1 ppm.” It was noted that cold-pressed citrus oils, which generally contain high levels of total furocoumarins, would need to be distilled prior to use in cosmetic products.

Lemon oil cold pressed (CAS RN 8008-56-8, 84929-31-7 and "any other CAS number(s) used to identify this fragrance ingredient"):

Implementation dates: For new submissions*: February 10, 2021

For existing fragrance compounds*: February 10, 2022

*These dates apply to the supply of fragrance mixtures (formulas) only, not to the finished consumer products in the marketplace.

Recommendation: Restriction

RESTRICTION LIMITS IN THE FINISHED PRODUCT (%):

| | | | |
|---|---|---|---|
| Category 1 | 2.0 % | Category 7A | No Restriction |
| Category 2 | 2.0 % | Category 7B | 2.0 % |
| Category 3 | 2.0 % | Category 8 | 2.0 % |
| Category 4 | 2.0 % | Category 9 | No Restriction |
| Category 5A | 2.0 % | Category 10A | No Restriction |
| Category 5B | 2.0 % | Category 10B | 2.0 % |
| Category 5C | 2.0 % | Category 11A | No Restriction |
| Category 5D | 2.0 % | Category 11B | 2.0 % |
| Category 6 | 2.0 % | Category 12 | No Restriction |

“The Standard is set due to the phototoxic effects of Lemon oil cold pressed. For more detailed information on the application of this Standard, please refer to the note on phototoxic ingredients in chapter 1 of the Guidance for the use of IFRA Standards.

If the level of furocoumarins is unknown, the restriction level specified in this IFRA Standard applies.

Combination effects of phototoxic ingredients are only taken into consideration for the furocoumarin-containing fragrance ingredients (extracts) listed in the IFRA Standard of Citrus oils and other furocoumarins containing essential oils.

If combinations of furocoumarin-containing phototoxic fragrance ingredients (extracts) are used, the use levels must be reduced accordingly. The sum of the concentrations of all furocoumarin containing phototoxic fragrance ingredients (extracts), expressed in % of their recommended upper concentration level in the consumer product shall not exceed 100.

For qualities of the expressed oil in which the less volatile components have been concentrated by partial or total removal of the terpene fraction, this limit should be reduced in proportion to the degree of concentration."

"Flavor requirements: Due to the possible ingestion of small amounts of fragrance ingredients from their use in products in Categories 1 and 6, materials must not only comply with IFRA Standards but must also be recognized as safe as a flavoring ingredient as defined by the IOFI Code of Practice (www.iofi.org). For more details see chapter 1 of the Guidance for the use of IFRA Standards."

"Intrinsic property driving risk management: phototoxicity."

"RIFM summaries: These recommendations are based on results of RIFM on the phototoxicity of lemon oil cold pressed (Fd. Cosm. Toxicol. 12,725 (1974), its low bergapten content (C.K. Shu et al. VI Int. Congress of Essential oils 1974) and the observed no-effect level of pooled samples in tests using the animal model."

"Expert Panel for Fragrance Safety rationale / conclusion: The Expert Panel for Fragrance Safety reviewed all the available data for Lemon oil cold pressed and recommends the limits for the 12 different product categories, which are the acceptable use levels of Lemon oil cold pressed in the various product categories."

As taken from IFRA, 2020a

IFRA restrictions for citrus oils and other furocoumarins containing essential oils:

RECOMMENDATION: RESTRICTION

| | | | |
|---|---|---|---|
| Category 1 | 0.0015 % (5-MOP) | Category 7A | No Restriction |
| Category 2 | 0.0015 % (5-MOP) | Category 7B | 0.0015 % (5-MOP) |
| Category 3 | 0.0015 % (5-MOP) | Category 8 | 0.0015 % (5-MOP) |
| Category 4 | 0.0015 % (5-MOP) | Category 9 | No Restriction |
| Category 5A | 0.0015 % (5-MOP) | Category 10A | No Restriction |
| Category 5B | 0.0015 % (5-MOP) | Category 10B | 0.0015 % (5-MOP) |
| Category 5C | 0.0015 % (5-MOP) | Category 11A | No Restriction |
| Category 5D | 0.0015 % (5-MOP) | Category 11B | 0.0015 % (5-MOP) |
| Category 6 | 0.0015 % (5-MOP) | Category 12 | No Restriction |

"The Standard is set due to the phototoxic effects of Citrus oils and other furocoumarins containing essential oils. For more detailed information on the application of this Standard, please refer to the note on phototoxic ingredients in chapter 1 of the Guidance for the use of IFRA Standards.

Where the Bergapten (5-Methoxypsoralen, (5-MOP)) content of all relevant oils present in a compound has been determined, it is recommended that for applications on areas of skin exposed to UV-light, the total level of Bergapten in the consumer products should not exceed 0.0015% (15 ppm). This upper concentration level only applies to applications on skin exposed to UV-light, excluding rinse-off products and incidental skin contact products as detailed in the Guidance for the use of IFRA Standards.

Where the level of Bergapten has not been determined by appropriate methods, the limits specified in the guidelines on individual oils should apply. In those cases, where such oils are used in combination with other furocoumarin-containing phototoxic fragrance ingredients (extracts), the additive effect has to be taken into consideration and the concentration levels have to be reduced accordingly.

The sum of the concentrations of all furocoumarin-containing phototoxic fragrance ingredients (extracts), expressed in % of their recommended upper concentration level in the finished consumer product, shall not exceed 100. Restrictions for furocoumarin-containing fragrance ingredients (extracts) have been recommended for:

• Angelica root oil, • Bergamot oil expressed, • Bitter orange oil expressed, • Cumin oil, • Grapefruit oil expressed, • Lemon oil cold pressed, • Lime oil expressed, • Rue oil.

The following essential oils contain small amounts of phototoxic furocoumarins (typical levels are provided in brackets):

• Petitgrain Mandarin oil (50 ppm), • Tangerine oil cold pressed (50 ppm), • Parsley leaf oil (20 ppm).

These levels are not high enough to require special restrictions if used alone, but if used in combination with one or the other furocoumarin-containing phototoxic fragrance ingredients (extracts), attention should be paid that the total level of Bergapten in the consumer product does not exceed 15 ppm."

"Flavor requirements: Due to the possible ingestion of small amounts of fragrance ingredients from their use in products in Categories 1 and 6, materials must not only comply with IFRA Standards but must also be recognized as safe as a flavoring ingredient as defined by the IOFI Code of Practice (www.iofi.org). For more details see chapter 1 of the Guidance for the use of IFRA Standards."

"Intrinsic property driving risk management: phototoxicity."

"RIFM summaries: These recommendations are based on the published phototoxic effects of Bergapten and the established dose-effect relationships (Young at al., J. Photochem. Photobiol. B,7, 231 (1990); Dubertret et al.ibid 7, 251 (1990), idem, ibid, 7, 362 (1990)."

"Expert panel for fragrance safety rationale / conclusion: The Expert Panel for Fragrance Safety reviewed all the available data for Citrus oils and other furocoumarins containing essential oils and recommends the limits for the 12 different product categories, which are the acceptable use levels of Citrus oils and other furocoumarins containing essential oils in the various product categories."

As taken from IFRA, 2020b

Citrus limon fruit oil (CAS RN 8008-56-8), Citrus limon peel oil (CAS RN 8008-56-8/84929-31-7) and Citrus limon flower extract, bud extract, flower water, callus culture extract and fiber (no CAS RNs given) are covered under Annex II/358 (List of substances prohibited in cosmetic products – Furocoumarines (e.g. trioxysalen (INN), 8-methoxypsoralen, 5-methoxypsoralen) except for normal content in natural essences used) of the EU cosmetic regulation. As taken from CosIng (Cosmetic substances and ingredients database). Available at https://ec.europa.eu/growth/tools-databases/cosing/, accessed May 2022.

Oils, lemon (CAS RN 8008-56-8) and Citrus medica limonum (lemon) fruit extract (no CAS RN given) are pre-registered under REACH ("envisaged registration deadline 31 May 2013"; "envisaged registration deadline 31 May 2018", respectively) (ECHA).

Lemon oil (CAS RN 8008-56-8), "no public or meaningful name is available" (CAS RN 8008-56-8; EC no. 934-840-2 and 935-455-2), lemon alcoholic extract, lemon essential oil, lemon ext. concentrated fraction, lemon extract and citrus lemon concentrated macerate (all with no CAS RNs given) are not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2022).

Lemon oil (CAS RN 8008-56-8) is listed in the US EPA Toxic Substances Control Act (TSCA) inventory and also in the US EPA 2020 CDR list (Chemical Data Reporting Rule). The CDR regulation 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 2020 CDR list are available at https://sor.epa.gov/sor_internet/registry/substreg/LandingPage.do

Lemon oil (Citrus limon (L.) Burm. F.) (CAS RNs 8008-56-8 and 84929-31-7), petitgrain lemon oil (Citrus limon L. Burm. F) (CAS RN 8008-56-8) and lemon essence oil (Citrus limon (L.) Burm. F.) (CAS RN 8008-56-8) have been given GRAS (generally recognized as safe) status by FEMA (FEMA Nos 2625, 2853 and 4852, respectively) (Cohen et al. 2018, 2019; Hall RL and Oser BL, 1965).

Oils, lemon (CAS RN 8008-56-8) are listed in the New Zealand Inventory of Chemicals does not have an individual approval but may be used under an appropriate group standard (NZ EPA, 2006) and are classified according to the Environmental Protection Authority of New Zealand (NZ EPA CCID).

Lemon oil (CAS RN 8008-56-8) and essence lemon (no CAS RN given) are included on the US FDA's list of inactive ingredients for approved drug products. They are permitted for use as ingredients in various products, at the following maximum potencies per unit dose and maximum daily exposure (MDE):

| Inactive Ingredient | Route | Dosage Form | CAS Number | UNII | Maximum Potency per unit dose | Maximum Daily Exposure (MDE) |
|---|---|---|---|---|---|---|
| ESSENCE LEMON | ORAL | SYRUP | | NA | 2.5mg | |
| LEMON OIL, COLD PRESSED | DENTAL | PASTE, DENTIFRICE | 8008568 | I9GRO824LL | 0.7%w/w | |
| LEMON OIL, COLD PRESSED | ORAL | CAPSULE | 8008568 | I9GRO824LL | 8.5mg | |
| LEMON OIL, COLD PRESSED | ORAL | SUSPENSION | 8008568 | I9GRO824LL | | 1mg |
| LEMON OIL, , COLD PRESSED | TOPICAL | GEL | 8008568 | I9GRO824LL | 0.05%w/w | |

As taken from FDA, 2022c

Lemon essential oil (no CAS RN given) is classified as an NHP [natural health product] under Schedule 1 item 2 (extract) of the NHP Regulations (Health Canada, 2022).

## *4. Metabolism/Pharmacokinetics*

### *4.1. Metabolism/metabolites*

"Citrus, one of the most popular fruits worldwide, contains various functional components, including flavonoids, dietary fibers (DFs), essential oils (EOs), synephrines, limonoids, and carotenoids. The functional components of citrus attract special attention due to their health-promoting effects. Food components undergo complex biotransformation by host itself and the gut microbiota after oral intake, which alters their bioaccessibility, bioavailability, and bioactivity in the host body. To better understand the health effects of citrus fruits, it is important to understand the in-vivo biotransformation of citrus functional components. We reviewed the biotransformation of citrus functional components (flavonoids, DFs, EOs, synephrines, limonoids, and carotenoids) in the body from their intake to excretion. In addition, we described the importance of biotransformation in

terms of health effects. This review would facilitate mechanistic understanding of the health-promoting effect of citrus and its functional components, and also provide guidance for the development of health-promoting foods based on citrus and its functional components." As taken from Karn A et al. 2021. Crit. Rev. Food Sci. Nutr. 61(5), 756-776. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32255367/

### *4.2. Absorption, distribution and excretion*

No data available to us at this time.

### *4.3. Interactions*

“LEMON OIL HAS BEEN REPORTED TO PROMOTE TUMOR FORMATION ON THE SKINS OF MICE TREATED WITH THE PRIMARY CARCINOGEN, 7,12-DIMETHYLBENZ[A]ANTHRACENE. /EXPRESSED LEMON OIL/ [Opdyke, D.L.J. (ed.). Monographs on Fragrance Raw Materials. New York: Pergamon Press, 1979., p. 491] **PEER REVIEWED**

As taken from HSDB, 2002

“Despite the widespread belief that citrus fruit extracts (CFEs) are microbiologically safe due to their acidity, limited bactericidal effect results in low applicability as antibacterial agent and outbreaks occurred by acid-adapted pathogens. Here, we examined the antibacterial effects of CFEs [lime (Citrus medica), lemon (Citrus limon), calamansi (Citrus microcarpa)] combined with essential oil components (EOCs; carvacrol and thymol) against non-acid-adapted/acid-adapted Escherichia coli O157:H7, Salmonella Typhimurium, and Listeria monocytogenes under 22 °C for 5 min. CFEs (<20%) alone or small amounts of EOCs (2.0 mM; 0.032%) alone could not inactivate the target bacteria effectively. However, combined treatments exhibited marked synergy: CFE + EOCs eliminated all the bacteria (>6.9 log CFU/ml). Among the CFEs tested, the highest synergism was shown by calamansi, an exotic citrus fruit previously unrecognized as an antibacterial agent. Although acid-adaptation improved bacterial survival, calamansi (<20%) + EOCs (<0.032%) completely inactivated even the most resistant pathogen (E. coli O157:H7). Validation test also showed that all tested commercial juice products also eliminated acid-adapted pathogens when used with EOCs. Physicochemical analysis of tested CFEs (pH measurement and HPLC analysis of components) revealed that low pH and flavanone (hesperidin) did not contribute to the synergistic bactericidal effects. Rather, the high citric acid content is likely to contribute to the strong synergistic effect with EOCs by damaging susceptible bacterial membranes. Sensory scores for CFEs were not altered by addition of EOCs at concentrations up to 1.5 mM. This study provides new insight into the utility of CFEs with EOCs to improve not only the microbiological safety of food products containing CFEs but also their applicability as natural antibacterial complex.” As taken from Chung D et al. 2018. Food Res. Int. 107, 578-588. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29580522

“The present study is planned to examine the antioxidant activity (AA) and the protective effect of the essential oil of Citrus limon (EOC) against aspirin-induced histopathological changes in the brain, lung, and intestine of female rats. For this purpose, 28 albino rats were classified to control group (group C), aspirin group (group A), EOC group (group EOC), and pretreatment with EOC and treated with aspirin group (group EOC + A). The antioxidant activities of EOC were evaluated by three different assays including reducing power, β-carotene, and scavenging of hydrogen peroxide (H2O2). Our results found that EOC represents, respectively (0.064 ± 0.013 and 0.027 ± 00 mg Quer E/100 µL), of flavonoid and flavonol. Then, it exhibited a potential activity of reducing power (at 300 mg/mL, which was found to be 0.82 ± 0.07), β-carotene-linoleic acid (AA% = 69.28 ± 3.5%), and scavenging of H2O2 (IC50 = 0.23 ± 0.008 mg/mL). In vivo, aspirin given to rats at the dose of 600 mg/kg body weight induced histomorphological damage in brain, lung, and intestine. However, our data found that the pretreatment with EOC offered a significant protection against the injury induced by aspirin. It can be concluded that the protective effect of EOC can be due to its

antioxidant activities." As taken from Bouzenna H et al. 2019. Hum. Exp. Toxicol. 38(5), 499-509. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30565488

"In recent years, herbal tea consumption becomes popular because of the potential health benefits and attractive flavors. However, there is also a growing concern that herbal supplements contribute to the drug-drug/drug-herb interactions and hepatotoxicity. In this study, FL83B mouse hepatocytes were used as an in vitro mode of hepatotoxicity induced by free fatty acids, including palmitic acid (PA) and oleic acid (OA), ethanol, and acetaminophen. Herbal tea extracts were obtained from eight common herbal plants, including Verbena officinalis L., Hyssopus officinalis L., Salvia officinalis L., Urtica dioica L., Hemerocallis fulva (L.) L., Citrus maxima (Burm.) Merr., Citrus limon (L.) Osbeck, and Ficus formosana Maxim. MTT assay was used to evaluate the impact of these herbal tea extracts on hepatoxocitity. We found that these herbal tea extracts per se did not exhibit hepatotoxicity, and had no effect on OA-induced hepatotoxicity. However, extracts from Verbena officinalis L., Hyssopus officinalis L., Salvia officinalis L., and Hemerocallis fulva (L.) L. exhibited protective effect against PA-induced hepatotoxicity. In addition, herbal tea extracts from Verbena officinalis L., Hyssopus officinalis L., Salvia officinalis L., Urtica dioica L., Hemerocallis fulva (L.) L., and Ficus formosana Maxim. exhibited protective effect against acetaminophen-induced hepatotoxicity. Interestingly, all these herbal tea extracts enhanced ethanol-induced hepatotoxicity. Our results suggest that herbal tea extracts have differential effects on different modes of hepatotoxicity." As taken from Chen G-W et al. 2019. Drug Chem. Toxicol. Epub ahead of print. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31736373/

## *5. Toxicity*

### *5.1. Single dose toxicity*

Oral LD50 (rat) and dermal (rabbit): >5 g/kg bw for (Hart, 1971; Levenstein, 1975).

Oral LD50 (rat): 2840 mg/kg bw (Anon, 1959).

Mice exposed to air containing lemon oil had a shortened barbiturate-induced sleeping time. Exposure presumably lasted until the mice awoke. The concentration was not specified, and the mechanism of the effect uncertain [possibly indicative of CNS excitation] (Tsuchiya et al., 1991).

### *5.2. Repeated dose toxicity*

No data available to us at this time.

### *5.3. Reproduction toxicity*

No data available to us at this time.

### *5.4. Mutagenicity*

In vivo:

No data available to us at this time

In vitro:

| Test system | Test conditions | Endpoint | Activation | Result | References |
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| Chinese hamster lung fibroblasts | Lemon oil tested at up to 0.125 mg/ml (the highest non-toxic concentration) in a 24-hr incubation, cells examined for chromosome aberrations | Chromosome damage and changes in chromosome number | Without | -ve (a limited study, as not tested with activation) | Ishidate et al., 1984 |

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|---|---|---|---|---|---|
| | and polyploidy. | | | | |
| Chinese hamster lung fibroblasts | Lemon oil tested at up to 0.125 mg/ml (the highest non-toxic concentration) in a 48-hr incubation, cells examined for chromosome aberrations. | Chromosome damage | Without | -ve (a limited study, as not tested with activation) | Ishidate et al., 1988 |
| Salmonella typhimurium TA98, TA100 | Ames test was used to screen 2 essential oil extracts of lemon [as well as 2 soluble essences and 2 hydroalcoholic extracts; no further details]. Tested at up to toxic concentrations or to a maximum of 100 ul/plate. | Mutation | With and without S9 | -ve (a limited study, only tested in 2 strains) | Crebelli et al., 1990 |
| Bacillus subtilis strains H17 and M45 | Tested at up to 30 ul/plate in a rec assay measuring differential killing. Information from tabulated data in a paper in Japanese. | DNA damage (indicative test) | With and without S9 | -ve (without S9) No result (with S9) [no toxicity in either strain] | Kuroda et al., 1989 |
| | Lemon oil has been recommended for photogenotoxicity testing by the NTP Interagency Committee for Chemical Evaluation and Coordination. | | | | PHS & DHHS, 2000 |

Lemon oil (CAS RN 8008-56-8) was negative in the bacterial mutagenicity (Ames) assay when tested, in the absence and presence of rat and hamster metabolic activation systems, in Salmonella typhimurium strains TA97, TA98, TA100 and TA1535 at concentrations of up to 333 µg/plate (NTP).

“The photogenotoxicity and photocarcinogenicity of several linear furocoumarins (psoralens) have been well documented (reviewed by Saffran, 1988). 5-Methoxypsoralen, a component of lemon and lime oil, is photogenotoxic in both prokaryotic and eukaryotic test systems. In addition, 5-methoxy-psoralen has been found to be photocarcinogenic in an experimental model (Zajdela & Bisagni, 1981). Little is known about the photogenotoxicity and photocarcinogenicity of other furocoumarins found in lemon oil and lime oil” (NIEHS, 2000).

“The applicant submitted in vitro genotoxicity tests performed with the additive under assessment, lemon extract (C. limon (L.) Osbeck). In order to investigate the potential of lemon extract to induce gene mutations in bacteria, the Ames test was performed according to OECD Test Guideline 471 (1997) and following Good Laboratory Practice (GLP) in 35 Lemon extract was tested in two independent experiments applying the plate incorporation method and the pre-incubation assay in the presence and absence of metabolic activation (S9-mix). Based on the results of a preliminary cytotoxicity assay, six concentrations ranging from lg/plate were selected to evaluate the induction of gene mutations. No increase in the mean number of revertant colonies was observed at any tested concentration in any tester strains with or without S9-mix. The FEEDAP Panel notes that the high toxicity of the test item limits the validity of the test. An in vitro micronucleus test was performed according to OECD TG 487 (2016) and following GLP to evaluate the potential of lemon

extract to induce chromosome damage in the absence and presence of metabolic activation.36 No significant cytotoxicity was observed at the three concentrations ( ) tested for the analysis of micronuclei after short treatments (3+ 21 h recovery) in the presence and absence of metabolic activation as well as continuous treatment (24 + 0 h recovery) in the absence of metabolic activation. The frequencies of micronuclei detected after treatment with lemon extract were comparable to the values observed in the concurrent negative controls. Therefore, the FEEDAP Panel concluded that lemon extract did not induce structural and numerical chromosomal aberrations in mammalian cells under the experimental conditions applied in the study."

As taken from EFSA, 2021a.

### *5.5. Cytotoxicity*

"In order to exploit novel anticaries agents, we investigated the effects of citrus lemon oil (CLO), a type of natural product, on growth and adherence of the primary oral cariogenic bacteria Streptococcus mutans (S. mutans). The growth inhibitory effect was explored with a micro-dilution assay. Adherence was analyzed by colony counts on the respective surfaces and the adherence inhibition rate (AIR). Real time-PCR was used to investigate the effects of CLO on transcription of glucosyltransferase (Gtf) encoding genes, gtfB, C and D. Neson-Somogyi method was used to measure the effects of CLO on Gtf activity. The minimum inhibitory concentration of CLO against S. mutans was 4.5 mg/ml. The CLO effectively reduced the adherence of S. mutans on glass surface (the AIR were from 98.3 to 100 %, $P > 0.05$) and saliva-coated enamel surface (the AIR were from 54.8 to 79.2 %, $P < 0.05$). CLO effectively reduced the activity of Gtf and the transcription of gtfs in a dose dependent manner ($P < 0.05$). In conclusion, CLO can effectively inhibit the growth and the adherence to glass and saliva-coated enamel surfaces of S. mutans. It can also inhibit the transcription of gtfs, as well as the Gtf enzyme activity." As taken from Liu Y et al. 2013. World J. Microbiol. Biotechnol. 29(7), 1161-7. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23381618?dopt=AbstractPlus

"Alicyclobacillus acidoterrestris is considered to be one of the important target microorganisms in the quality control of acidic canned foods. There is an urgent need to develop a suitable method for inhibiting or controlling the germination and outgrowth of A. acidoterrestris in acidic drinks. The aim of this work was to evaluate the chemicals used in the lemon industry (sodium benzoate, potassium sorbate), and lemon essential oil as a natural compound, against a strain of A.acidoterrestris in MEB medium and in lemon juice concentrate. The results pointed out that sodium benzoate (500-1000-2000 ppm) and lemon essential oil (0.08-0.12-0.16%) completely inhibited the germination of A. acidoterrestris spores in MEB medium and LJC for 11 days. Potassium sorbate (600-1200 ppm) was more effective to inhibit the growth of the microbial target in lemon juice than in MEB medium. The effect of sodium benzoate, potassium sorbate and essential oil was sporostatic in MEB and LJC as they did not affect spore viability." As taken from Maldonado MC et al. 2014. Braz. J. Microbiol. 44(4), 1133-7. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24688502

"Candida yeasts are saprophytes naturally present in the environment and forming colonies on human mucous membranes and skin. They are opportunistic fungi that cause severe and even fatal infections in immunocompromised individuals. Several essential oils, including eucalyptus, pine, cinnamon and lemon, have been shown to be effective against Candida strains. This study addresses the chemical composition of some commercial lemon essential oils and their antifungal potential against selected Candida yeast strains. Antifungal potential and minimum inhibitory concentrations were determined for six commercial lemon essential oils against five Candida yeast strains (Candida albicans 31, Candida tropicalis 32, Candida glabrata 33, Candida glabrata 35 and Candida glabrata 38). On the basis of the GCMS analysis, it was found that the tested lemon essential oils had different chemical compositions, but mostly, they contained almost exclusively terpenes and oxygenated terpenes. The tests show that antifungal potential of lemon essential oils against Candida yeast strains was related to the high content of monoterpenoids and the type of Candida strains. From six tested commercial oils, only four (ETJA, Vera-Nord, Avicenna-Oil and

Aromatic Art) shows antifungal potential against three Candida species (C. albicans, C. tropicalis and C. glabrata). Vera-Nord and Avicenna-Oil show the best activity and effectively inhibit the growth of the C. albicans strain across the full range of the concentrations used. Our study characterises lemon essential oils, which could be used as very effective natural remedies against candidiasis caused by C. albicans." As taken from Biaéoä M et al. 2014. Mycopathologia 177(1-2), 29-39. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24436010

"Salads prepared from contaminated fresh produce have a high risk of causing food-borne illnesses. Essential oils obtained from plants have antimicrobial activity and may provide a natural approach to reduce the pathogens on fresh produce. Additionally, ultrasound treatments have been shown to reduce the microbial counts on different foods. The objective of this study was to investigate the antimicrobial activities of cinnamon and lemon essential oils in vitro and in food applications. Mixtures of lettuce, parsley and dill were inoculated with Listeria monocytogenes and then dip-treated for 5 min in one of the following treatments: sterile tap water, chlorinated water, 1% lemon essential oil, 2% cinnamon essential oil or 2% cinnamon essential oil + ultrasound. The samples were stored at 4 (°C) and collected at d 0, 1, 3, 5, 7 and 9 post inoculation. The 1% lemon (4 log) and 2% cinnamon (2 log) essential oil washes provided partial inhibition against L. monocytogenes by d 1. The combined application of 2% cinnamon oil and ultrasound resulted in only 0.85 log inhibition by d 1; however, the number of L. monocytogenes increased during storage and became nearly equal to the control at d 9. Therefore, different combinations of essential oils with other antimicrobials or novel technologies are required." As taken from Özcan G and Demirel Zorba NN. 2016. Food Sci. Technol. Int. 22(4), 353-62. PubMed, 2017 available at

"The aim of the present study was to assess the antifungal and anti-toxinogenic activity of 15 essential oils (EOs) against three fungi of the genus Aspergillus (A. parasiticus KMi-227-LR, A. parasiticus KMi-220-LR and A. flavus KMi-202-LR). The minimum inhibitory doses (MIDs) of the tested essential oils and their antifungal activity were determined using the micro-atmosphere method. The original commercial essential oil samples of Jasminum officinale L., Thymus vulgaris L., Syzygium aromaticum (L.) Merrill & Perry, Rosmarinus officinalis L., Ocimum basilicum L., Eucalyptus globulus Labill., Salvia officinalis L., Citrus limon (L.) Burm, Origanum vulgare L., Lavandula angustifolia Mill., Carum carvi L., Citrus sinensis (L.) Osbeck., Zingiber officinalis Rosc., Mentha piperita L. and Cinnamomum zeylanicum Nees. (C. verum J.S.Presl.) were produced in Slovakia (Calendula a.s., Nová -ubova, Slovakia). All essential oils exhibited activity against all tested strains of fungi. After 14 days of incubation, A. flavus (KMi-202-LR) showed the highest susceptibility with a growth inhibition percentage (GIP) of 18.70% to C. limon and 5.92% to C. sinensis, while A. parasiticus (KMi-220-LR) exhibited a GIP of 20.56% to J. officinale. The minimum inhibitory doses (MIDs) of EOs with the most significant activity were recorded. The best antifungal activity, using the micro-atmosphere method was found in S. aromaticum with an MID of 62.5 µL L-1 air, T. vulgaris (MID of 62.5 µL L-1 air) and O. vulgare (MID of 31.5 µL L-1 air) against all tested strains. Mycotoxin production of the tested strains was evaluated by the thin layer chromatography (TLC) method. Mycotoxin production of AFB1 and AFG1 was inhibited following all treatments with C. carvi, R. officinale and S. officinale, Eucalyptus globulus L. and O. basilicum L. Essential oils exhibited a potential inhibition activity against toxic fungi, although, these affected only the production of AFB1." As taken from Císarová M et al. 2016. J. Environ. Sci Health B 51(10), 668-674. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27322876

"Natural plant extracts contain a variety of phenolic compounds which are assigned various biological activities. Our work aims to make a quantitative and qualitative characterization of the Zest (ZL) and the Flesh (FL) of lemon (Citrus limon), to valorize the pharmacological uses of lemon, by evaluating in vitro activities (DPPH, free radical scavenging and reducing power). The antibacterial, antifungal, and antiproliferative activities were sought in the ability of Citrus limon extracts to protect DNA and protein. We found that the ZL contains high amounts of phenolics responsible for the important antioxidant properties of the extract. However, the FL is richer in flavonoids than the ZL. The FL extract was also found to be more effective than the ZL in protecting plasmid DNA against the strand breakage induced by hydroxyl radicals. We also concluded that the

FL extract exhibited potent antibacterial activity unlike ZL. Analysis by LC/MS-MS identified 6 compounds (Caffeoyl N-Tryptophan, Hydroxycinnamoyl-Oglucoside acid, Vicenin 2, Eriocitrin, Kaempferol-3-O- rutinoside, and Quercetin-3-rutinoside). These preliminary results showed that Citrus limon has antibacterial and antioxidant activity in vitro. It would be interesting to conduct further studies to evaluate the in vivo potential in an animal model." As taken from Makni M et al. 2018. Biomed. Res. Int. 2018, 6251546. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29568760

"OBJECTIVES: Lemon essential oil (LEO) is a kind of secondary metabolite from lemon peels and has been found to inhibit cariogenic bacteria for decades. However, its effects on main cariogenic virulence factors are rarely reported. The present study aimed to investigate the effects of sub-minimum inhibitory concentrations (sub-MICs) of LEO on the acid tolerance and biofilm formation of Streptococcus mutans (S. mutans) and preliminarily reveal the possible underlying mechanisms. DESIGNS: Effects of LEO on the acid tolerance and biofilm formation of S. mutans were investigated by the broth dilution method and crystal violet staining method respectively. The expression of luxS, srtA and spaP gene was also determined to explore the underlying mechanism. In addition, Tea polyphenols (TP), a major natural inhibitor of cariogenic virulence factors, and limonene (LIM), the major component of LEO, were selected as comparisons to evaluate the effects of LEO. RESULTS: Sub-MICs of LEO, LIM and TP exhibited a dose-dependent inhibition of growth of S. mutans at pH ranging from 4.0 to 7.0. The formation of S. mutans biofilm was remarkably inhibited and the inhibitory rates of LEO, LIM and TP were 97.87%, 94.88% and 96.01% respectively at 1/2 MIC. Similarly, a down-regulation was observed in the expression of luxS, srtA and spaP gene at sub-MIC levels. CONCLUSIONS: Effects of LEO were similar or slightly stronger than LIM and TP, suggesting that LEO might represent a novel, natural anticarious agent that inhibited the specific genes associated with bacterial acid tolerance and biofilm formation without necessarily affecting the growth of oral bacteria." As taken from Sun Y et al. 2018. Arch. Oral Biol. 87, 235-241. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29331510

"OBJECTIVES: The chemical composition, antimicrobial and synergistic effect, and cytotoxic activity of Citrus limon (lemon), Piper nigrum (green pepper) and Melaleuca alternifoila (tea tree) essential oils (EOs) were investigated. METHODS: Chemical analyses of essential oils were tested by GC-FID and GC-MS spectroscopy. The antimicrobial activity assay was conducted using microdilution method against several oral bacteria and Candida spp. originating from the humans with oral disorders. The synergistic antimicrobial activity was evaluated using checkerboard method. The cytotoxicity evaluation of EOs was assessed using MTT test. KEY FINDINGS: Limonene (37.5%) and β-pinene (17.9%) were the major compounds in C. limon oil, β-pinene (34.4%), δ-3-carene (19.7%), limonene (18.7%) and α-pinene (10.4%) in P. nigrum oil and terpinen-4-ol (38.6%) and γ-terpinene (21.7%) in M. alternifolia oil. The broad-spectrum antimicrobial activity was achieved by tested three EOs, with C. limon oil being the strongest against bacteria and M. alternifolia oil strongest against fungi. The EOs demonstrated synergism; their combined application revealed an increase in antimicrobial activity. All tested essential oils showed lower cytotoxic activity in comparison with the positive control, and the obtained results confirmed a dose-dependent activity. CONCLUSIONS: The results of this study encourage use of tested EOs in development of a novel agent intended for prevention or therapy of corresponding oral disorders." As taken from Nikoli-ç MM et al. 2017. J. Pharm. Pharmacol. 69(11), 1606-1614. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28815601

"BACKGROUND: Lemon (Citrus limon) is a flowing plant belonging to the Rutaceae family. Citrus plants constitute one of the main valuable sources of essential oil used in foods and medicinal purposes. METHODS: In this study, we assessed chemical composition, antioxidant and antimicrobial activities of C. limon essential oil (ClEO) with its preservative effect against Listeria monocytogenes inoculated in minced beef meat. Gas chromatography/mass spectrometry (GC-MS) was used to identify the major components of the obtained ClEO. The antioxidant activities of this ClEO were determined according to the β-carotene bleaching assay, as well as by 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. For antimicrobial activity, agar well

diffusion method was used and the minimum inhibitory concentrations (MICs) as well as the minimum fungicidal concentrations (MFCs) were determined. The in situ effect of the ClEO was evaluated through physicochemical parameters (pH and thiobarbituric acid reactive substances (TBARS), as well as against L. monocytogenes in minced beef meat model. RESULTS: Twenty one components were identified in the ClEO and the two dominant compounds were limonene (39.74%) and β-Pinene (25.44%). This ClEO displayed an excellent DPPH scavenging ability with an extract concentration providing 50% inhibition (IC50) of 15.056 µg/ml and a strong β-carotene bleaching inhibition after 120 min of incubation with an IC50 of 40.147 µg/ml. The MICs varied from 0.039 to 1.25 mg/ml for Gram positive bacteria and from 0.25 to 2.5 mg/ml for Gram-negative bacteria. The meat preserving potential of ClEO was investigated against L. monocytogenes. ClEO successfully inhibited development of L. monocytogenes in minced beef meat. The application of ClEO at a 0.06 and 0.312 mg/g, may open new promising opportunities for the prevention of contamination from and growth of pathogenic bacteria, particularly L. monocytogenes, during minced beef meat storage at 4 °C. Additionally, during storage period, physicochemical values (pH and TBARS) were higher in control meat than treated meat with ClEO suggesting an efficient antioxidant activity of ClEO. CONCLUSION: It was suggested that the ClEO may be a new potential source as natural antimicrobial and antioxidant agents applied in food systems and pharmaceutical industry." As taken from Ben Hsouna A et al. 2017. Lipids Health Dis. 16(1), 146. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28774297

"In the present study, soil samples were collected from hospital nearby areas and some bacteria were isolated and purified by using spread plate and streak plate methods. The six strains named A, B, C, D, E and F were cultured. Cultural, morphological and biochemical characterization was done to identify theses bacterial isolates. Eighteen Indian medicinal plants were selected to examine the antimicrobial activity against the cultured species using disk diffusion method. Methanolic extraction as well as water extract of the plant leaves and roots was prepared and phytochemical screening of these plants was performed for constituents such as flavonoids, saponins, glycosides, tannins, phytosteroid, phenols, alkaloids, etc. Among all the plants Citrus Limon showed maximum antimicrobial activity (by inhibition zone method) against the isolated microbes. However, the isolated microbes were susceptible to the methanolic extracts of the above mentioned plants. In future, these plants can be further subjected to isolation of the therapeutic antimicrobials for health purposes and food applications." As taken from Jangra MR et al. 2018. Int. J. Pure App. Biosci. 6(1), 461-466. Available at http://www.ijpab.com/form/2018%20Volume%206,%20issue%201/IJPAB-2018-6-1-461-466.pdf

"In developing country herbal medicine used widely to treat infectious disease. The Citrus limon peel rich in nutrient such as flavonoid and essential oil that can used for antimicrobial and anticancer activity. The Citrus limon peel extracted with ethanol 96% using maceration extraction and then dissolved with DMSO. The antimicrobial activity tested against Escherichia coli using disc diffusion assay. The present study shows the antimicrobial activity of the ethanolic extract of the Citrus limon peel of 25%, 50%, 75% and 100% had a strong antimicrobial activity against Escherichia coli were 15,03 mm; 16,17 mm; 15,83 mm; 18,77mm of the average inhibition zone, respectively. The essential oil can disrupt the bacterial membrane and damage the lipids and proteins layer. The flavonoid can inhibit specific enzymes and scavenge free radical. It concluded that lemon (Citrus limon) peel extract have a high potential on antimicrobial activity against the Escherichia coli." As taken from Henderson AH et al. 2018. American Scientific Research Journal for Engineering, Technology, and Sciences 39(1), 268-273. Available at http://www.asrjetsjournal.org/index.php/American_Scientific_Journal/article/view/3805

"In view of the increasing interest in natural antimicrobial molecules, this study screened the ability of Thymus capitatus (TC) essential oil and Citrus limon var. pompia (CLP) extract as raw extracts or incorporated in vesicular nanocarriers against Streptococcus mutans and Candida albicans. After fingerprint, TC or CLP were mixed with lecithin and water to produce liposomes, or different ratios of water/glycerol or water/propylene glycol (PG) to produce glycerosomes and penetration

enhancer vesicles (PEVs), respectively. Neither the raw extracts nor the nanovesicles showed cytotoxicity against human gingival fibroblasts at all the concentrations tested (1, 10, 100 µg/mL). The disc diffusion method, MIC-MBC/MFC, time-kill assay, and transmission electron microscopy (TEM) demonstrated the highest antimicrobial potential of TC against S. mutans and C. albicans. The very high presence of the phenol, carvacrol, in TC (90.1%) could explain the lethal effect against the yeast, killing up to 70% of Candida and not just arresting its growth. CLP, rich in polyphenols, acted in a similar way to TC in reducing S. mutans, while the data showed a fungistatic rather than a fungicidal activity. The phospholipid vesicles behaved similarly, suggesting that the transported extract was not the only factor to be considered in the outcomes, but also their components had an important role. Even if other investigations are necessary, TC and CLP incorporated in nanocarriers could be a promising and safe antimicrobial in caries prevention." As taken from Pinna R et al. 2019. Pharmaceutics 11(5), E234. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/31091818

"Hepatitis C is a serious health issue and cause liver disorders in millions of people. Available therapeutic agents require long term administration with numerous side effects. Therefore, there is a dire need to find alternative treatment options for this disease. Since ancient times, medicinal plants are widely used to cure various diseases with no or less harmful effects. Therefore, this study was designed to find out phytochemicals and investigate antiviral activity of methanol extract of Ajuga bracteosa, Ajuga parviflora, Berberis lycium and Citrus lemon against Hepatitis C Virus (HCV infection). Phytochemical analysis of the plant extract was performed using various chemical tests. Toxicity of the plant extract was determined against using trypan blue exclusion method. Antiviral activity of the selected plant extract was find out against HCV infected HepG2 cells. For this purpose, HepG2 cells were seeded with HCV positive and negative serum and nontoxic doses of plant extract for 24 and 48 h. After this RNA was extracted and viral load was determined using Real-time PCR. Phytochemical analysis showed the presence of flavonoids and phenols in all plant extracts while amino acids, alkaloids and tannins were present in B. lycium and saponins were detected in C. lemon. Toxicity assay showed that all plant extracts were nontoxic at maximum concentration of 200 µg/ml except B. lycium, which showed mild toxicity at 40 µg/ml and were extremely toxic at 60 µg/ml and above doses. Real-time PCR quantitation result revealed that after 24 h treatments A. parviflora showed highest antiviral activity, followed by A. bracteosa, while B. lycium extract had low (35%) and C. lemon has no antiviral effects. The 48 h treatments showed an increase antiviral activity by A. bracteosa followed by A. parviflora and B. lycium while C. lemon showed negative effect. Our results depicted that mentioned plants might be used as an alternative therapeutic regime or in combination with existing treatments against HCV." As taken from Yousaf T et al. 2018. Microb. Pathog. 118, 154-158. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29571723

"The in vitro cytotoxic activity on human cancer cell lines of sixteen commercial EOs such as Aloysia citriodora, Boswellia sacra, Boswellia serrata, Cinnamomum zeylanicum, Cistus ladanifer, Citrus × aurantium, Citrus limon, Citrus sinensis, Cymbopogon citratus, Foeniculum vulgare, Illicium verum, Litsea cubeba, Satureja montana, Syzygium aromaticum, Thymus capitatus and Thymus vulgaris was performed using the MTT reduction assay. The screening was carried out on human cancer cells of breast adenocarcinoma (MCF7, T47D and MDA-MB-231), chronic myelogenous erythroleukemia (K562) and neuroblastoma cell lines (SH-SY5Y). C. zeylanicum and L. cubeba EOs were the most active on almost all the cell lines studied and thus could be promising as an anticancer agent. These two species showed a difference in their composition even though they belong to the Lauraceae family. Almost 57 % of the true cinnamon composition was made of (E)-cinnamaldehyde, while L. cubeba showed citral as the major compound (68.9 %). The K562 cells were the most sensitive to these oils with an IC50 ranging from 5.2 parts-per million (ppm) (C. zeylanicum) to 11.1 ppm (L. cubeba). The latter oil also showed an important cytotoxicity on MDA-MB-231 (13.4 ppm)." As taken from Najar B et al. 2020. Chem. Biodivers. 17(1), e1900478. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31713998/

“Objective: The main objective of the study was to evaluate the cytotoxicity of selected essential oils on human skin, gastric, and brain cancer cell lines using microculture tetrazolium test. Materials and methods: Phytochemical analysis, as well as acute oral toxicity tests, was carried out in female albino mice with cardamom oil, lemon oil, and jasmine oil according to the Organization for Economic Co-operation and Development guidelines 425. Anticancer activities of the above test drugs were performed using human cancer cell lines. The studies were carried out at Skanda Life Sciences Pvt. Ltd., Bengaluru. Results: Phytochemical analysis has shown the presence of carbohydrates and flavonoids in cardamom oil. While lemon oil has shown the presence of carbohydrates, flavonoids, steroids, terpenoids, and tannins, jasmine oil has shown the presence of carbohydrates, alkaloids, flavonoids, steroids, terpenoids, and glycosides. Toxicity studies showed that cardamom oil, lemon oil, and jasmine oil were all found to be safe up to 2000 mg/kg body weight. Results have shown that lemon oil exhibited the strongest cytotoxicity toward three human cancer cell lines, namely skin cancer (A431), gastric cancer (MKN-45), and brain cancer (U-87 MG) cell lines, with higher $IC_{50}$ values of 62.82 µg/ml, 220.9 µg/ml, and 440.1 µg/ml compared to standard. Jasmine oil exhibited the strongest cytotoxicity toward skin cancer and brain cancer cell lines, whereas cardamom oil has shown stronger cytotoxicity only toward skin cancer cell line but did not show any level of inhibition of growth of brain and gastric cancer cells. Conclusion: Our study reveals that lemon oil, jasmine oil, and cardamom oil possess potent antitumor activity compared to standard. At different concentrations, lemon oil has shown statistically significant (***P < 0.0001) anticancer activity toward all the three human cancer cell lines. While jasmine oil has shown statistically significant (***P < 0.0001) anticancer activity toward skin and brain cancer cell line, cardamom oil has also shown statistically significant (***P < 0.0001) anticancer activity but only toward skin cancer cell line.” As taken from Manjunath C and Mahurkar N. 2021. J. Cancer Res. Ther. 17(1), 62-68. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33723134/

“Leishmaniasis is a tropical parasitic disease that affects up to 12 million people worldwide. Current chemotherapies have limitations such as toxicity, high cost, and parasite resistance. This work aims to select an essential oil (EssOil) isolated from the Tunisian flora as a new antileishmanial candidate. Two plants were chosen for their antileishmanial potential: Citrus limon (Citrus) and Pistacia lentiscus (Pistacia). Each of these plants was harvested from two different sites (area 1 and area 2). Extracted EssOils were characterized using GC-MS. Their antiparasitic activity against axenic and intracellular Leishmania major amastigotes and their cytotoxicity were assessed. Citrus EssOil from area 1 displayed an interesting activity against L. major intramacrophage amastigotes with $IC_{50}$ value at 4.2 ± 1.3 µg/mL. Interestingly, this activity was close to that of miltefosine. Moderate activities against intracellular amastigote were observed for Pistacia EssOil from area 1 and Citrus EssOil from area 2. However, low cytotoxicity with high selectivity index was proved only for Citrus EssOil from area 1, revealing its safety for macrophages. This study also demonstrated for the first time the antileishmanial activity of EssOil extracted from Citrus limon leaves. The EssOil interesting activity could be related to the lipophilic properties of terpenes that were shown in literature to contribute to the disruption of parasite intracellular metabolic pathways.” As taken from Maaroufi Z et al. 2021. Parasitol Res. 120(4), 1455-1469. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33426571/

“Aim: Anxiety and intolerance to dental local anesthetic injections are common in patients undergoing dental procedures. This work was designed to study cytotoxicity of selected flavors in primary gingival keratinocytes (PGK), to acquire information on their suitability for use in dental lidocaine hydrochloride (LID) injection. We also evaluated the bio-mimetic taste of LID dental injection in the presence of selected flavors and sweetener using an Astree electronic tongue (ETongue). Methods: The cytotoxicity of chocolate natural and artificial flavor (CTE), raspberry flavor artificial (RAS), cherry flavor (CHR), bitterness suppressor flavor (BSF) and lemon flavor extract (LFE) at various dilutions (0.16-10% v/v) was carried out in PGK using the live cell morphological analysis and MTT cell cytotoxicity assay. Based on the cytotoxicity data, CTE and RAS were added to Xylocaine® (2%) along with 0.09% sodium saccharin and taste was assessed using an ETongue. Results: After three hours of treatment, a dose-dependent cell death was

induced by all flavors compared to the untreated control. BSF was found to be more toxic when compared to other flavors. CTE was found to be less toxic. The mean IC50 values of CTE, RAS, CHR, BSF and LFE in PGK were found to be 9.54, 8.43, 2.21, 0.38 and 4.01 mg/mL. Taste analysis with the ETongue showed a clear taste difference between the control and test formulations containing CTE and RAS flavors along with sodium saccharin. Conclusion: CTE and RAS flavors in combination with 0.09% sodium saccharin can achieve a significant taste-masking effect in the dental LID injection." As taken from Boddu SHS et al. 2020. Pharmaceuticals (Basel) 13(11), 353. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33138130/

### *5.6. Carcinogenicity*

| **Species** | **Test conditions** | **Evidence of carcinogenicity** | **Reference** |
|---|---|---|---|
| Mouse | Tumour promotion assay.<br>Weekly application for 33 wk of 0.25 ml [ca. 12 g/kg bw/week] lemon oil to uncovered skin previously treated with a skin carcinogen | Some evidence of tumour promotion, as lemon oil increased the ability of the carcinogen to produce benign skin tumours | Roe & Peirce, 1960 |
| Rat & mouse, groups of 50 per sex per species | Though no carcinogenicity studies on lemon oils were identified, high quality studies have been carried out by the US NTP on d-limonene (at 80-90% the major constituent of lemon oil). This major component was given by gavage to rats and mice on 5 days/wk for 2 years. Comprehensive tissue examinations were carried out. | No evidence in mice or in female rats. Kidney tumours developed in male rats.<br>It was JECFA's view that the mechanism of tumour induction was probably not relevant to humans (JECFA, 1993a). | NTP, 1990; JECFA, 1993b |
| | Lemon oil has been recommended by the NTP for photocarcinogenicity testing, pending the results of photogenotoxicity tests. | | PHS & DHHS, 2000 |

"The photogenotoxicity and photocarcinogenicity of several linear furocoumarins (psoralens) have been well documented (reviewed by Saffran, 1988). 5-Methoxypsoralen, a component of lemon and lime oil, is photogenotoxic in both prokaryotic and eukaryotic test systems. In addition, 5-methoxy-psoralen has been found to be photocarcinogenic in an experimental model (Zajdela & Bisagni, 1981). Little is known about the photogenotoxicity and photocarcinogenicity of other furocoumarins found in lemon oil and lime oil" (NIEHS, 2000).

Lemon oil has been reported to promote tumor formation on the skin of mice by the primary carcinogen 9,10-dimethyl-l,2-benzanthracene.

As taken from Khan IA and Abourashed EA, 2010.

Lemon oil (CAS RN 8008-56-8) is a suspected carcinogen. The CAESAR carcinogenicity model and the ISS carcinogenicity model in the VEGA (Q)SAR platform predict that the chemical is a carcinogen (EXPERIMENTAL value).

As taken from ECHA, 2021

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

| Route/Organism | Dose | Effect | Reference |
|---|---|---|---|

| | | | |
|---|---|---|---|
| administration onto the skin, mouse | TDLo: 280 gm/kg/33W-I | TUMORIGENIC: Equivocal tumorigenic agent by RTECS criteria; SKIN AND APPENDAGES: Other: Tumors; TUMORIGENIC: Tumors at site of application; | Journal of the National Cancer Institute. (Washington, DC) V.1-60, 1940-78. For publisher information, see JJIND8. 24,1389,1960 |

As taken from RTECS, 2008

“BACKGROUND: Citrus comprises the largest fruit sector worldwide, and its fruit peels are the dominant 'residue' of the industry. Though not profitable, Citrus peels are industrially used for making some byproducts (cattle feed, molasses, ethanol, fiber) and for the extraction of bioactives (flavonoids, essential oils, d-limonene). Still huge amounts of peels are wasted by Citrus industries, juice and other vending sectors. PURPOSE: The biological potentials of these unutilized or 'wasted' Citrus peels are least exploited. Here we tested the anticancer potentials of Citrus medica (2 morphotypes), C. sinensis, C. maxima, C. limon and C. reticulata peels by in vitro assays and in vivo cancer models. METHODS: Chemical profiles of Citrus peel oils and peel extracts were analyzed by gas chromatographic techniques (GC-FID, GC-MS) and HPTLC-densitometry, respectively. Anticancer potentials of Citrus peels (Citrus medica 2 morphotypes, C. sinensis, C. maxima, C. limon and C. reticulata) were evaluated by various in vitro assays (MTT assay, morphological observations, fast halo assay, flow cytometric analysis) and in vivo cancer models. RESULTS: C. reticulata peels (extracts, essential oils) showed significant activity against DLA cell line in MTT assay. We found C. reticulata peel water extract inducing cell cycle arrest of DLA in G0/G1 phase followed by nuclear condensation, membrane blebbing, formation of apoptotic bodies and DNA damage leading to apoptosis. In in vivo experiments, C. reticulata peel extract pre-treated mice were significantly (50%) protected from DLA compared to post-treated mice (33%), without any conspicuous toxic symptoms. Citrus peels have volatiles (essential oils, limonoids) and non-volatiles (mainly polymethoxy flavones) as their bioactive/anticancer constituents. CONCLUSION: Our results encourage the use of Citrus peels, which is wasted in huge amounts, as cancer preventive food additives and as anticancer agents.” As taken from Nair SA et al. 2018. Phytomedicine 50, 231-237. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30466983

“OBJECTIVE: To quantify the relationship between Citrus intake and risk of cancer of the oral cavity and pharynx. DESIGN: Systematic review and meta-analysis. DATA SOURCES: Ovid MEDLINE, EMBASE, and Web of Science were searched until September 2017. Search terms included Citrus, Citrus aurantifolia, Citrus sinensis, Citrus paradisi, Citrus fruits, Citrus fruits extract, Citrus oil, fruits, oral cancer, mouth cancer, mouth neoplasm. STUDY SELECTION: The selection of studies and the systematic review were carried out in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. A pre-defined inclusion checklist resulted in the inclusion of articles which were (i) published in peer-reviewed scientific journals; (ii) English language; (iii) and included a measure of Citrus fruit intake and risk of oral and pharyngeal cancer. Studies were excluded if (i) preparations derived from other fruits were used, (ii) Citrus intake was combined with intake of other fruits; (iii) in vitro or animal models were used. We also excluded reviews, systematic reviews, meta-analyses, letters, personal opinions, conference abstracts and book chapters. DATA EXTRACTION: Three reviewers independently performed the extraction of data from studies included. RESULTS: Seventeen studies met our inclusion criteria and were included in the final review. Pooled analyses showed that those with the highest Citrus fruit intake compared to the lowest intake had a 50% reduction in risk of oral cavity and pharyngeal cancer (OR 0.50; 95% CI 0.43-0.59). CONCLUSION: The studies included in this review and meta-analysis showed an inverse association between Citrus fruit intake and oral cancer.” As taken from Cirmi S et al. 2018. Pharmacol. Res. 133, 187-194. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29753688

*5.7. Irritation/immunotoxicity*

“Lemon oil applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was moderately irritating (Hart, 1971)” (Opdyke, 1974a & b).

“Applied full strength to intact or abraded rabbit skin for 24 hr under occlusion, it [lemon petitgrain oil] was not irritating (Levenstein, 1975)” (Opdyke, 1978).

“Three samples of lemon oil … applied undiluted to the backs of hairless mice were mildly irritating (Urbach & Forbes, 1972), but three other samples of lemon oil … similarly applied … were not irritating (Urbach & Forbes, 1972)” (Opdyke, 1974a).

“Lemon oil distilled … applied undiluted to the backs of hairless mice was slightly irritating (Urbach & Forbes, 1974). Lemon oil FCF applied undiluted to the backs of hairless mice was not irritating (Urbach & Forbes, 1974)” (Opdyke, 1974b).

“Undiluted lemon petitgrain oil applied to the backs of hairless mice and swine was slightly irritating (Urbach & Forbes, 1972)” (Opdyke, 1978).

“Lemon oil tested at 10% in petrolatum produced no irritation after a 48-hr closed-patch test in 25 human subjects (Kligman 1971)” (Opdyke, 1974a & b).

“A patch test using full strength lemon oil for 24 hr produced no reactions in 24 human subjects (Katz, 1946)” (Opdyke, 1974a).

Tested at 10% in petrolatum, [lemon petitgrain oil] produced no irritation after a 48-hr closed-patch test on human subjects (Epstein, 1975)” (Opdyke, 1978).

Volunteers exposed to 450 mg/m^(3) d-limonene for 2 hours showed no signs of respiratory irritation (Von Burg, 1995).

“The aim of this study was to investigate the causes of skin diseases in one-third of the staff of a perfume factory, in which 10 different perfume sprays were being manufactured. It involved site inspections, dermatological examinations and patch testing of all 26 persons at risk with four perfume oils and 30 of their constituents. Six bottlers were found to be suffering from allergic contact dermatitis, two from irritant contact dermatitis and 12 showed strong reactions to various fragrances. The main causes of allergic contact dermatitis were two perfume oils (12 cases) and their ingredients geraniol (12 cases), benzaldehyde (9), cinnamic aldehyde (6), linalool, neroli oil, terpenes of lemon oil and orange oil (4 each). None tested positive to balsam of Peru. Proposed measures to reduce the risks included changes to jobs with no contact with fragrances, automation, replacement of glass bottles by cartridges from non-fragile materials and use of gloves” (Schubert, 2006).

| Route/Organism | Dose | Effect | Reference |
|---|---|---|---|
| administration onto the skin, mouse | 100% | mild irritant effect | Food and Cosmetics Toxicology. (London, UK) V.1-19, 1963-81. For publisher information, see FCTOD7. 12,727,1974 |
| administration onto the skin, rabbit | 500 mg/24H | moderate irritation effect | Food and Cosmetics Toxicology. (London, UK) V.1-19, 1963-81. For publisher information, see FCTOD7. 12,725,1974 |

As taken from RTECS, 2008

**Skin and respiratory tract sensitization**

At 10% lemon oil and lemon petitgrain oil were not sensitising to human skin (Epstein, 1975; Kligman, 1971).

The literature contains a brief report of a woman who developed allergy-related cough, headache and sore throat symptoms following a 10-minute exposure to the vapour from an open cup containing 0.1% “citrus peel oils” in isopropanol. At 1%, symptoms were more severe and reduced lung function was noted (Block & Marney, 1988). [The source of the oils may have been orange

peel, since the woman's original symptoms (nasal and bronchial congestion) resulted from occupational exposure to an orange-scented room deodorizer.]

Contact dermatitis and eczema patients were patch tested with lemon oil at 2% in petrolatum. None of the 48 patients tested exhibited definite positive reactions (Guin, 2005).

Two patients with contact dermatitis, associated with the use of essential aromatherapy oils, were patch-tested with 2% lemon oil in petrolatum. One negative and one strong positive reaction were observed (Trattner et al., 2008).

The results of patch-tests on 15,682 dermatitis patients were analysed. Of these, 6467 patients had been patch-tested with lemon oil at 2% in petrolatum. 0.22% of individuals exhibited a weakly positive sensitisation reaction, with stronger reactions seen in 0.07% (Uter et al., 2010).

"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... 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…. The Rudzki 1976 study found 1 positive reaction in 200 patients to "lemon" essential oil 2% pet…. The later Rudzki 1986 study in 86 FM I positive patients found n=2 (2.3%) positive reactions to "lemon" essential oil 2% pet…. The IVDK 2010 c study identified 0.3% positive reactions in 6467 patients tested in the context of a special series…".

As taken from SCCS, 2012.

"Available data from one source indicate lemon oil to be nonirritating and nonsensitizing to human skin, though some samples have been demonstrated to be irritating to the backs of hairless mice… Lemon petitgrain oil has been reported to be nonirritating, nonsensitizing, and nonphototoxic to human skin."

As taken from Khan IA and Abourashed EA, 2010."Nearly 80 essential oils (including 2 jasmine absolutes) have caused contact allergy. Fifty-five of these have been tested in consecutive patients suspected of contact dermatitis, and nine (laurel, turpentine, orange, tea tree, citronella, ylang-ylang, sandalwood, clove, and costus root) showed greater than 2% positive patch test reactions. Relevance data are generally missing or inadequate. Most reactions are caused by application of pure oils or high-concentration products. The clinical picture depends on the responsible product. Occupational contact dermatitis may occur in professionals performing massages. The (possible) allergens in essential oils are discussed. Several test allergens are available, but patients should preferably be tested with their own products. Co-reactivity with other essential oils and the fragrance mix is frequent, which may partly be explained by common ingredients. Patch test concentrations for essential oils are suggested." As taken from de Groot AC and Schmidt E. 2016. Dermatitis 27(4), 170-175. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27427818

Lemon oil (CAS RN 8008-56-8) is a suspected skin sensitizer. The CAESAR skin sensitization model in the VEGA (Q)SAR platform predicts that the chemical is a sensitizer (EXPERIMENTAL value).

As taken from ECHA, 2021

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

"Immediate skin reactions are common in dermatological practice, but may often be overlooked. The main objective of this article is to provide an update of the literature concerning immediate-type reactions or contact urticaria/contact urticaria syndrome caused by cosmetic ingredients in terms of immediate clinical symptoms, positive reactions following open, scratch or, most often, prick testing,

and sometimes the detection of specific IgE antibodies. To this end, a selective search in different medical literature databases was performed. This yielded a list of cosmetic ingredients causing immediate reactions, including hair dyes and bleaches, preservatives, fragrance and aroma chemicals, sunscreens, hair glues, plant-derived and animal-derived components, permanent makeup and tattoos, glycolic acid peel, lip plumper, and alcohols. Many of the reported cases, however, lack appropriate controls and detailed investigation. Contact urticaria may occur with or without systemic symptoms, which are sometimes life-threatening." As taken from Verhulst L and Goosens A. 2016. Contact Dermatitis 75(6), 333-344. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27593503

"BACKGROUND: Gold lotion (GL), a natural mixed product made from the peels of six citrus fruits, has recently been identified as possessing anti-oxidative, anti-inflammatory, and immunomodulatory effects. GL has been used to protect skin against UV-induced damage, but its activity against psoriasis, a chronic autoimmune skin disease caused by dysregulation between immune cells and keratinocytes, is not known. We therefore evaluated the effect of GL on imiquimod (IMQ)-induced psoriasis-like inflammation in mice. RESULTS: GL treatment significantly attenuated IMQ-induced psoriasis-like symptoms in mice. The inflammatory cytokines upregulated by IMQ in skin lesions were also inhibited by feeding GL. In addition, GL treatment reduced the infiltration of CD4+ T cells/neutrophils in skin lesions and the percentage of IL-17-/IL-22-producing T cells in lymph nodes. Furthermore, GL impaired IMQ-induced type I interferon production by plasmacytoid dendritic cells (pDCs) in vitro. CONCLUSION: Our results indicate GL can act to suppress the initiation of psoriasis and strongly suggest that GL may have potential to be applied to the treatment of psoriasis." As taken from Lin CC et al. 2018. J. Sci. Food Agric. 98(14), 5509-5517. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29691866

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

"Background: Naturally derived cosmetic product ingredients of both plant and animal origin are being included increasingly in product formulations in order to cater to consumer preferences. They may be an overlooked cause of reactions to cosmetic products in some patients with dermatitis. Objectives: To identify naturally derived cosmetic product ingredients with allergenic potential (type I and type IV) and propose a cosmetic screening test series. Methods: The study was conducted in two steps. The first step was a market survey using a nonprofit application helping consumers avoid problematic substances in cosmetic products. The application contained 10 067 cosmetic products that were label checked for naturally derived cosmetic product ingredients. The second step was a literature search to examine how frequently the naturally derived ingredients were described and related to allergic reactions in cosmetics or other topically administered products. Results: We identified 121 different naturally derived cosmetic product ingredients that were included in at least 30 cosmetic products. In total, 22 ingredients were selected for a screening test series. Conclusions: We propose a supplemental patch test and a prick test screening series with naturally derived cosmetic product ingredients for patients with skin reactions to cosmetic products,

aiming to identify a cause in more patients than is currently possible." As taken from Bruusgaard-Mouritsen MA et al. 2020. Contact Dermatitis 83(4), 251-270. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32248558/

"Background: Several studies have shown anti-inflammatory, anti-microbial, antifungal, and antioxidant effects from Citrus limon-peel essential oil (Cl-PEO). Cl-PEO can be developed as topical drugs for oral ulceration because of its potential active components. There have been no studies on the topical application of Cl-PEO inducing type IV hypersensitivity reaction. Purpose: To investigate the potential of Cl-PEO from Batu City to induce type IV hypersensitivity reactions based on clinical changes, lymphocytes, macrophages, IFNγ, andIL10 expression. Methods: This study was adapted from a guinea pig maximization-test method in Indonesia and the guidance of ISO 10,993-10:2010, and conducted on 20 guinea pigs (Cavia cobaya) divided into a control group and a treatment group. The treatment group was given Cl-PEO and the control group CMC-Na. Clinical changes were observed, then tissue specimens taken for hematoxylin-eosin and immunohistochemistry staining. Results: There were no clinical changes after exposure. Lymphocyte and macrophage numbers and IFNγ and IL10 expression increased in the treatment group compared to the control group (p=0). Conclusion: Cl-PEO can induce type IV hypersensitivity reactions in guinea pigs based on cellular and molecular cytokines, but there are no clinical changes after topical application." As taken from Mahdani FY et al. 2020. J. Exp. Pharmacol. 12, 213-220. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32801948/

"No specific data were provided by the applicant regarding the safety of the additive for users. The low pH of the additive (< 4) would indicate a likelihood of skin and eye irritation, and potentially corrosion. The additive should be considered a skin and eye irritant, and potential corrosive."

As taken from EFSA, 2021a.

No specific data were provided by the applicant regarding the safety of the additive for users.The applicant produced a safety data sheet55for lemon oil'distilled'(combined residual and distilledfractions of expressed lemon oil) where hazards for users have been identified. Since lemon oil expressed contains furocoumarin derivatives, the additive may cause phototoxicity (NTP, 2000).

As taken from EFSA, 2021b

Background: In about half of the patients reacting positive to fragrance mix I (FM I),breakdown testing remains negative. This raises the question of whether the reactionto FM I is false-positive, or the breakdown test is false-negative.

Objectives:To identify characteristics and sensitization patterns of patients positive to FM I, but not to its fragrance constituents.

Patients and Methods: Retrospective analysis of data from the Information Networkof Departments of Dermatology (IVDK) between 2005 and 2019. Three patient groups were defined according to their reaction pattern: Group I, FM I positive and≥1 single fragrance positive in the breakdown test (n = 1912); Group II, FM I positive and breakdown test negative (n = 1318); Group III, FM I negative (n = 19 790).

Results: Regarding the pattern of concomitant reactions to other fragrances, Group II had an intermediate position between Group I and Group III. In other respects (ageand sex distribution, frequency of sensitization to non-fragrance baseline series aller-gens), Group II rather resembled Group I.

Conclusions: Not every positive reaction to FM I in patients with negative break-down tests is false-positive. There may be false-negative reactions to the single fra-grance components when patch tested at 1% pet. Raising patch concentrations ofsome single fragrances is recommended.

As taken from Geier et al. 2021.

### *5.8. All other relevant types of toxicity*

Low-level to distinct phototoxic effects were reported with lemon oil, depending on the origin of the oil (Urbach & Forbes, 1972).

No phototoxic effects were reported with lemon oil distilled (Urbach & Forbes, 1972), lemon oil FCF (Urbach & Forbes, 1974) or lemon petitgrain oil (Urbach & Forbes, 1972).

“The aim of this study, linked-up with a previous study on bergamot oils, was the evaluation of phototoxic potential of essential oils (orange, lemon and Litsea cubeba), used as cosmetic ingredients. The applied tiered testing strategy included chemical analysis of the substances (by means of capillary gas chromatography/mass spectrometry), in vitro 3T3 NRU phototoxicity test and EpiDerm™ skin phototoxicity test. In order to clarify the situation in man, the highest non-phototoxic/non-cytotoxic concentrations and concentrations 10 x lower (safety factor 10) were tested xin vivo by means of human skin photopatch test in a limited group of human volunteers. The study revealed, that phototoxicity of the essential oils was dependent on the content of photoactive components and the solvent used. The highest non-phototoxic concentrations obtained by the skin model assay proved to be a useful starting point for subsequent confirmatory human photopatch test aimed to identify safe concentration for human use. However, the highest non-phototoxic concentration obtained in the skin model assay cannot be applied directly for human practice (3 of 8 tested oils evoked a phototoxic reaction). A safety factor of 10 should be applied for extrapolation of experimental data from the skin model assay to man” (Kejlová et al., 2010).

“Lemon oil was diluted in ethanol (100% lemon oil, 50% lemon oil, and 20% lemon oil) and applied to backs of shaved, albino guinea pigs. Animals were then exposed to UVA radiation (320-400 nm, 13 J/cm2). Erythema was evaluated at 24, 48 and 72 hr after irradiation. Undiluted lemon oil and 50% lemon oil elicited phototoxicity for most of the samples tested. Lemon oil from Australia and Brazil elicited a weaker phototoxic than lemon oil from the Ivory Coast, Sicily, California, or Argentina. In an effort to identify the phototoxic components in lemon oil, these investigators fractionated samples by solvent extraction and subsequent phototoxicity testing of isolated components. Two phototoxic components were identified: oxypeucedanin and 5-methoxypsoralen” (NIEHS, 2000).

Lemon oil was fractionated and its phototoxicity was measured. The substances producing phototoxicity were identified as being oxypeucedanin and bergapten (Naganuma, 1985).

“This study sought to investigate the effects of essential oil from lemon (Citrus limoni) peels on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities in vitro. The essential oil was extracted by hydrodistillation, dried with anhydrous Na2SO4 and characterized using gas chromatography. Antioxidant properties of the oil and inhibition of pro-oxidant-induced lipid peroxidation in rats brain homogenate were also assessed. The essential oil inhibited AChE and BChE activities in a concentration-dependent manner. GC analysis revealed the presence of sabinene, limonene, α-pinene, β-pinene, neral, geranial, 1,8-cineole, linalool, borneol, α-terpineol, terpinen-4-ol, linalyl acetate and β-caryophyllene. Furthermore, the essential oil exhibited antioxidant activities as typified by ferric reducing property, Fe(2+)-chelation and radicals [DPPH, ABTS, OH, NO] scavenging abilities. The inhibition of AChE and BChE activities, inhibition of pro-oxidant induced lipid peroxidation and antioxidant activities could be possible mechanisms for the use of the essential oil in the management and prevention of oxidative stress-induced neurodegeneration.” As taken from Oboh G et al. 2014. J. Oleo. Sci. 63(4), 373-81. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24599102

“Lemon oil has been reported to have phototoxic effects, most likely due to its coumarins”.

“Lemon petitgrain oil has been reported to be nonirritating, nonsensitizing, and nonphototoxic to human skin.”

As taken from Khan IA and Abourashed EA, 2010.

“The aim of the present study was to assess the antifungal and anti-toxinogenic activity of 15 essential oils (EOs) against three fungi of the genus Aspergillus (A. parasiticus KMi-227-LR, A. parasiticus KMi-220-LR and A. flavus KMi-202-LR). The minimum inhibitory doses (MIDs) of the tested essential oils and their antifungal activity were determined using the micro-atmosphere method. The original commercial essential oil samples of Jasminum officinale L., Thymus vulgaris L., Syzygium aromaticum (L.) Merrill & Perry, Rosmarinus officinalis L., Ocimum basilicum L., Eucalyptus globulus Labill., Salvia officinalis L., Citrus limon (L.) Burm, Origanum vulgare L., Lavandula angustifolia Mill., Carum carvi L., Citrus sinensis (L.) Osbeck., Zingiber officinalis Rosc., Mentha piperita L. and Cinnamomum zeylanicum Nees. (C. verum J.S.Presl.) were produced in Slovakia (Calendula a.s., Nová -ubova, Slovakia). All essential oils exhibited activity against all tested strains of fungi. After 14 days of incubation, A. flavus (KMi-202-LR) showed the highest susceptibility with a growth inhibition percentage (GIP) of 18.70% to C. limon and 5.92% to C. sinensis, while A. parasiticus (KMi-220-LR) exhibited a GIP of 20.56% to J. officinale. The minimum inhibitory doses (MIDs) of EOs with the most significant activity were recorded. The best antifungal activity, using the micro-atmosphere method was found in S. aromaticum with an MID of 62.5 µL L-1 air, T. vulgaris (MID of 62.5 µL L-1 air) and O. vulgare (MID of 31.5 µL L-1 air) against all tested strains. Mycotoxin production of the tested strains was evaluated by the thin layer chromatography (TLC) method. Mycotoxin production of AFB1 and AFG1 was inhibited following all treatments with C. carvi, R. officinale and S. officinale, Eucalyptus globulus L. and O. basilicum L. Essential oils exhibited a potential inhibition activity against toxic fungi, although, these affected only the production of AFB1.” As taken from Císarová M et al. 2016. J. Environ. Sci Health B 51(10), 668-674. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27322876

“Citrus Limon Oil (CLO) is known as antioxidant resource and contains limonoids, flavonoids and phenolic compounds. This study was conducted to investigate the effects of different levels of CLO on blood parameters and antioxidant status in Afshari ewes. Six adults Afshari ewes (3-4 years old and 51±5 kg) were randomly allocated to 3×3 Latin square design with three diets in 21 days period. Dietary treatments included:1) control diet, 2) control diet with 200 mg/day CLO, and 3) control diet with 400 mg/day CLO. To evaluate the antioxidant effect of the CLO, sustainable elimination of free radicals by DPPH and ABTS methods were used. The antioxidant activity of essential oils in DPPH method at doses of 32.5, 45, 130, 260 and 520 mg/ml were 9, 16, 31, 49 and 89%, respectively. Also, antioxidant activity of essential oils in ABTS method at doses of 32.5, 45, 130, 260 and 520 mg/ml were 49, 73, 81, 89 and 95%, respectively. CLO treatments did not affect glucose, blood urea nitrogen, albumin, total protein, low density lipoprotein, while improved the concentration of high-density lipoprotein (P>0.01). Results showed that supplementation with CLO significantly decreased (P<0.01) cholesterol, triglycerides and very low density lipoprotein concentrations compared with control. There was no significant difference in analyzed blood bio-chemicals and serum enzymes level between different antioxidant activity methods and groups, suggesting general well-being of ewes. These results suggest that, CLO supplementation had a positive impact on blood traits and antioxidant status of the Afshari ewes.” As taken from Mojtahedin A et al. 2018. Cell. Mol. Biol. (Noisy-le-grand) 64(1), 47-51. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29412793

“The influence of natural, hot-air and infrared drying on chemical composition and bioactivity of lemon peel essential oil are investigated in this study. The results showed that drying resulted in losses or increases of some components or production of some new substances, but the d-limonene (59.52-70.01%) was found as the main component of essential oil. Drying brought about decreases in the yield, antioxidant and antibacterial activity of essential oil. However, the natural drying had little effect, while the hot-air and infrared drying resulted in significant decreases in these parameters, especially at the higher temperature. The yield was the lowest under hot-air drying (60 °C) and decreased by 78%, while infrared drying (60 °C) sample exhibited the lowest antioxidant and antibacterial activities. Infrared drying was easier to lead to the decrease in bioactivity than hot-air drying at the same temperature. These results provided the theoretical basis for drying lemon

peel." As taken from Zhang et al. 2017. Nat. Prod. Res. 32(10), 1184-1188. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28438032

Many of South Africans living in rural communities still depend heavily on medicinal plants for their healthcare needs. A recent ethnobotanical study done in some of the rural communities in northern Maputaland revealed that the lay people use medicinal plants (singularly and in plant combinations) for the treatment of hypertension. It is well known that traditional people do not only depend on a single plant extract for the treatment but they often combine various parts/species to increase the efficacy. Although a fair amount of research has been done on the toxicity of medicinal plants singularly, very little has been done on the toxicity of medicinal plants when used in combination. Thus, the aim of this study was to evaluate the safety of 26 medicinal plants and 19 plant combinations which were recorded for the treatment of hypertension. Only one combination (Aloe marlothii and Hypoxis hemerocallidea) tested has been previously investigated for toxicity. The aqueous extracts were assessed using the brine shrimp assay (Artemia franciscana). For the plants which were tested singularly, only Citrus limon and Catharanthus roseus were toxic. These two extracts were further tested at varied concentrations to determine ratio dosages. For the plant combinations, six combinations were toxic at 4 mg/ml. However, when tested at 2 mg/ml, only three combinations were toxic (A. marlothii with C. roseus; C. roseus with Musa acuminata; C. roseus with Momordica balsamina). The overall results indicated that toxicity was dosage dependant with combinations and low toxicity was observed for most plants administered independently, with the exception of C. roseus, which was toxic individually and when in combinations. As taken from Ramulondi M et al. (2018). In vitro toxicological evaluation of some antihypertensive plants. South African Journal of Botany 115, 307-308. Available at https://www.sciencedirect.com/science/article/pii/S0254629918303041

"This study analyzed the effects of the plant extracts (Citrus limon, Solanum lycopersicum, Zingiber officinale, Vitis vinifera and Allium sativum) on the growth of mammalian cells (Vero and MDA-MB-231) and evaluated the most effective plant extract for the expression of specific genes of the JAK/STAT pathway in human breast cancer cells. An antiproliferative bioassay involving neutral red-dye uptake was used to determine the anticancerous potential of plant extracts. In Vero cells, the ginger methanolic extract was least effective; whereas the lemon methanolic extract was more effective with 64 dilutions with IC50 51.42%. In MDA-MB-231 cells, the tomato and ginger methanolic, and grape water extracts were least effective, whereas lemon water extract was most effective with 32 dilutions with IC50 48.67%, by upregulating JAK1, JAK2, TYK2, IRF7 and IRF3 gene expressions of the JAK/STAT pathway. C. limon inhibited the growth of both Vero and MDA-MB 231 cells. It suggested that C. limon has anti-cancer potential by inducing the JAK/STAT pathway." As taken from Mahmood N et al. 2021. Nucleosides Nucleotides Nucleic Acids 20(4), 434-469. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33749513/

## *6. Functional effects on*

### *6.1. Broncho/pulmonary system*

The literature contains a brief report of a woman who developed allergy-related cough, headache and sore throat symptoms following a 10-minute exposure to the vapour from an open cup containing 0.1% "citrus peel oils" in isopropanol. At 1%, symptoms were more severe and reduced lung function was noted (Block & Marney, 1988). [The source of the oils may have been orange peel, since the woman's original symptoms (nasal and bronchial congestion) resulted from occupational exposure to an orange-scented room deodorizer.]

"OBJECTIVE: The aim of this pilot study is to analyze the reactions of healthy test persons and conscious as well as unconscious palliative patients to aroma stimuli. PATIENTS AND METHODS: In a randomized controlled study, healthy probands, conscious and unconscious palliative patients were exposed to two essential oils (lemon, lavender). Water was used as the control stimulus. Physiological parameters (breathing rate and heart rate, oxygen saturation, systolic, diastolic and

mean arterial pressure) were measured. RESULTS: 10 test persons (control group), 15 conscious patients and 5 unconscious palliative patients were exposed to the stimuli. Healthy test persons reacted to lemon oil with a significant increase in respiration rate, heart rate and diastolic blood pressure, whereas to lavender oil with a significant decrease in respiration rate was measured. There were no significant reactions concerning the other parameters. Conscious and unconscious patients reacted with a significant increase in all measured parameters to lemon oil and with a significant decrease in all parameters except for oxygen saturation to lavender oil. No significant differences in the reactions were measured between the conscious and unconscious patients and no significant reactions to control stimuli were detected. All physiological reactions were very short and only detectable during stimulus presentation. CONCLUSION: Significant physiological reactions were measured after simulation with aroma oils in all three groups in this study. Healthy probands showed different reactions than palliative patients irrespective to their conscious state." As taken from Goepfert M et al. 2017. J. Cancer Res. Clin. Oncol. 143(10), 2123-2129. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28634728

### *6.2. Cardiovascular system*

"OBJECTIVE: The aim of this pilot study is to analyze the reactions of healthy test persons and conscious as well as unconscious palliative patients to aroma stimuli. PATIENTS AND METHODS: In a randomized controlled study, healthy probands, conscious and unconscious palliative patients were exposed to two essential oils (lemon, lavender). Water was used as the control stimulus. Physiological parameters (breathing rate and heart rate, oxygen saturation, systolic, diastolic and mean arterial pressure) were measured. RESULTS: 10 test persons (control group), 15 conscious patients and 5 unconscious palliative patients were exposed to the stimuli. Healthy test persons reacted to lemon oil with a significant increase in respiration rate, heart rate and diastolic blood pressure, whereas to lavender oil with a significant decrease in respiration rate was measured. There were no significant reactions concerning the other parameters. Conscious and unconscious patients reacted with a significant increase in all measured parameters to lemon oil and with a significant decrease in all parameters except for oxygen saturation to lavender oil. No significant differences in the reactions were measured between the conscious and unconscious patients and no significant reactions to control stimuli were detected. All physiological reactions were very short and only detectable during stimulus presentation. CONCLUSION: Significant physiological reactions were measured after simulation with aroma oils in all three groups in this study. Healthy probands showed different reactions than palliative patients irrespective to their conscious state." As taken from Goepfert M et al. 2017. J. Cancer Res. Clin. Oncol. 143(10), 2123-2129. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28634728

"BACKGROUND: we have evaluated the antihypertensive effect of several flavonoid extracts in a rat model of arterial hypertension caused by chronic administration (6 weeks) of the nitric oxide synthesis inhibitor, L-NAME. METHODS: Sprague Dawley rats received L-NAME alone or L-NAME plus flavonoid-rich vegetal extracts (Lemon, Grapefruit + Bitter Orange, and Cocoa) or purified flavonoids (Apigenin and Diosmin) for 6 weeks. RESULTS: L-NAME treatment resulted in a marked elevation of blood pressure, and treatment with Apigenin, Lemon Extract, and Grapefruit + Bitter Orange extracts significantly reduced the elevated blood pressure of these animals. Apigenin and some of these flavonoids also ameliorated nitric oxide-dependent and -independent aortic vasodilation and elevated nitrite urinary excretion. End-organ abnormalities such as cardiac infarcts, hyaline arteriopathy and fibrinoid necrosis in coronary arteries and aorta were improved by these treatments, reducing the end-organ vascular damage. CONCLUSIONS: the flavonoids included in this study, specially apigenin, may be used as functional food ingredients with potential therapeutic benefit in arterial hypertension." As taken from Paredes MD et al. 2018a. Nutrients 10(4), E484. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29652818

"The cholesterol-lowering and anti-atherogenic effects of lemon essential oil (LEO) were investigated and compared with the effects of limonene. Owing to their volatility, both LEO and

limonene were microencapsulated before preparation of the diet (20%, w/w). Hypercholesterolemia-induced rabbits were divided into 3 groups based on plasma total cholesterol (TC) levels and fed coating matrix (control group), LEO (LEO group), or limonene (Limonene group) for 8 weeks. LEO dose-dependently inhibited low-density lipoprotein oxidation in vitro. Plasma TC levels were the lowest in the LEO group ($P<0.05$). Erythrocytes in the LEO group had a normal disc shape, whereas the erythrocytes in the limonene and control groups were aggregated and star-shaped, respectively. The aortic intima thickness was thinnest in the LEO group followed by the control and limonene groups. Plasma TC lowering and anti-atherogenic effects of LEO were greater than limonene, suggesting that other bioactive compounds besides limonene in LEO might contribute to these effects. The bioactive compounds in LEO were limonene (67.57%), β-pinene (10.00%), and γ-terpinene (9.95%). In addition, sabinene, α-pinene, myrcene, and geranial were also present but the amount was in the range of 1~2%. Several bioactive compounds were also detected. In conclusion, LEO had beneficial effects on hypercholesterolemia due to its antioxidative and cholesterol lowering effects." As taken from Lee H et al. 2018. Prev. Nutr. Food Sci. 23(1), 8-14. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29662842

"In the present study, the anti-platelet aggregation activity of 14 vegetables and fruits was tested in vitro. The aqueous, 90% ethanol and ethyl acetate extracts, as well as concentrated juices of 14 foods (fruits and vegetables) were prepared, and the anti-platelet aggregation activity of those extracts was analyzed on a platelet aggregation analyzer in vitro with adenosine 5'-diphosphate (ADP), bovine thrombin (THR) and arachidonic acid (AA) as aggregation inducers, respectively. Aspirin (ASP) was used as the positive control. A number of the tested foods had inhibitory effects in concentration-dependent manner on platelet aggregations induced by various agonists. Especially, some foods such as lemon, leek, garlic, scallion, ginger, tomato and grapefruit showed good anti-platelet aggregation effect similar or higher than that of positive control group i.e. aspirin (ASP). The results of present study provide scientific reference for reasonable selection of daily dietary with supplementary curative effects or prevention of cardiovascular diseases (CVD)." As taken from Chen C et al. 2019. Pak. J. Pharm. Sci. 32(1), 185-195. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30772808

"Many studies have shown that flavonoids are effective as antihypertensive drugs in arterial hypertension. In the present work, we have analyzed the effects of some flavonoid extracts in the spontaneous hypertensive rat model (SHR). An important feature of this study is that we have used a low dose, far from those that are usually applied in human therapy or experimental animals, a dose that responded to the criterion of a potential future commercial use in human subjects. Treatments were carried out for 6 and 12 weeks in two groups of SHR rats, which received apigenin, lemon extract, grapefruit + bitter orange (GBO) extracts, and cocoa extract. Captopril was used as a positive control in the SHR group treated for 6 weeks (SHR6) and Diosmin was used as the industry reference in the SHR group treated for 12 weeks (SHR12). Captopril and GBO extracts lowered the high arterial pressure of the SHR6 animals, but none of the extracts were effective in the SHR12 group. Apigenin, lemon extract (LE), GBO, and captopril also improved aortic vascular relaxation and increased plasma and urinary excretion of nitrites, but only in the SHR6 group. Kidney and urinary thiobarbituric acid reactive substances (TBARS) were also significantly reduced by GBO in the SHR6 rats. Apigenin also improved vascular relaxation in the SHR12 group and all the flavonoids studied reduced urinary thiobarbituric acid reactive substances (TBARS) excretion and proteinuria. Vascular abnormalities, such as lumen/wall ratio in heart arteries and thoracic aorta, were moderately improved by these treatments in the SHR6 group. In conclusion, the flavonoid-rich extracts included in this study, especially apigenin, LE and GBO improved vascular vasodilatory function of young adult SHRs but only the GBO-treated rats benefited from a reduction in blood pressure. These extracts may be used as functional food ingredients with a moderate therapeutic benefit, especially in the early phases of arterial hypertension." As taken from Paredes MD et al. 2018b. Nutrients 10(8), E1107. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30115889

"Background: This study aimed to determine the effect of lemon (Limon Citrus) inhalation aromatherapy on Blood Pressure (BP), electrocardiogram changes, and anxiety in patients with Acute Myocardial Infarction (AMI). Methods: In this clinical trial, 100 AMI patients were assigned to intervention (received lemon inhalation aromatherapy) and control (received paraffin) groups. Aromatherapy was conducted for three consecutive days. BP, electrocardiogram changes, and anxiety were assessed. Results: A significant difference was observed between the two groups concerning systolic BP and percentage of ST-segment and T wave changes on the 3rd and 4th days after the intervention. A significant difference was also found between the two groups concerning heart rate and anxiety on the 4th day after the intervention. Conclusion: As lemon inhalation aromatherapy in AMI reduced systolic BP, anxiety, and percentage of ST-segment and T wave changes and regulated heart rate, using this kind of aromatherapy is suggested in coronary care units." As taken from Rambod M et al. 2020. Complement. Ther. Clin. Pract. 39, 101155. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/32379635/

### *6.3. Nervous system*

Mice exposed to air containing lemon oil had a shortened barbiturate-induced sleeping time. Exposure presumably lasted until the mice awoke. The concentration was not specified, and the mechanism of the effect uncertain [possibly indicative of CNS excitation] (Tsuchiya et al., 1991).

Prolonged exposure to lemon essential oil (150 µl added to bedding every three days for a total of 11-12 days) resulted in increased anxiety and gender-specific (female only) change to formalin-induced pain responses in Sprague Dawley rats (20 per gender) (Ceccarelli et al., 2004).

“The anti-dementia effects of s-limonene and s-perillyl alcohol were observed using the passive avoidance test (PA) and the open field habituation test (OFH). These lemon essential oils showed strong ability to improve memory impaired by scopolamine; however, s-perillyl alcohol relieved the deficit of associative memory in PA only, and did not improve non-associative memory significantly in OFH. Analysis of neurotransmitter concentration in some brain regions on the test day showed that dopamine concentration of the vehicle/scopolamine group was significantly lower than that of the vehicle/vehicle group, but this phenomenon was reversed when s-limonene or s-perillyl alcohol were administered before the injection of scopolamine. Simultaneously, we found that these two lemon essential oil components could inhibit acetylcholinesterase activity in vitro using the Ellman method” (Zhou et al., 2009).

“BACKGROUND: AFFECTIONS ARE THOUGHT TO REGULATE PAIN PERCEPTION THROUGH THE DESCENDING PAIN INHIBITORY SYSTEM IN THE CENTRAL NERVOUS SYSTEM. IN THIS STUDY, WE EXAMINED IN MICE THE AFFECTIVE CHANGE BY INHALATION OF THE LEMON OIL, WHICH IS WELL USED FOR AROMATHERAPY, AND THE EFFECT OFLEMONODOR ON PAIN SENSATION. WE ALSO EXAMINED THE ANTERIOR CINGULATE CORTEX (ACC) AND DESCENDING PAIN INHIBITORY SYSTEM TO SUCH REGULATION OF PAIN. RESULTS: In the elevated plus maze, the time spent in the open arms was increased by inhalation oflemon oil. The pain behavior induced by injection of formalin into the hind paw was decreased. By inhalation oflemon oil, the number of c-Fos expression by formalin injection was significantly increased in the ACC, periaqueductal grey (PAG), nucleu raphe magnus (NRM) and locus ceruleus, and decreased in the spinal dorsal horn (SDH). The destruction of the ACC with ibotenic acid led to prevent the decrease of formalin-evoked nocifensive behavior in mice exposed tolemon oil. In these mice, the change of formalin-induced c-Fos expression in the ACC, lateral PAG, NRM and SDH bylemonodor was also prevented. Antagonize of dopamine D1 receptor in the ACC prevented to the analgesic effect oflemon oil. CONCLUSIONS: These results suggest that the analgesic effect oflemon oilis induced by dopamine-related activation of ACC and the descending pain inhibitory system.” As taken from Ikeda H et al. 2014. Mol. Pain 10, 14. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24555533

“Citrous lemon (Rutaceae) an Indian folk medicine has been used for the treatment of various pathological diseases viz., diabetes, cardiovascular, inflammation, hepatobiliary dysfunction and neurodegenerative disorder. Can lemon oil altered the memory of unstressed and stressed mice, a basic question for which the present work was put on trial. The present investigation was intended to assess the impact of Lemon oil on memory of unstressed and Stressed Swiss young Albino mice. Lemon oil (50 and 100 mg/kg o.r.) and donepezil (10 mg/kg) were guided for three weeks to different groups of stressed and unstressed mice. The nootropic movement was assessed utilizing elevated plus maze and Hebbs Williams Maze. Cerebrum acetylcholinesterase (AChE), plasmacorticosterone, decreased glutathione, lipid per oxidation alongside superoxide dismutase and catalase was surveyed as marker for disease. Histopathology was performed for estimation of drug effects. Acute immobilized stress was induce, lemon oil (100 mg/kg) and donepezil together indicated memory enhancing movement both in stressed and unstressed mice. Lemon oil significantly ($p < 0.001$) altered and lowered brain AChE activity both in stressed and unstressed mice. Scopolamine induced amnesia was also significantly altered and reversed both in stressed and unstressed mice by lemon oil at a dose of 50 and 100 mg/kg. Lemon oil (50 and 100 mg/kg) was further able to control the corticosterone level in plasma for stressed mice. Lemon oil significantly ($p < 0.001$) elevated the level of catalase, superoxide dismutase and reduced glutathione levels both in stressed and unstressed animals with respect to controlled group along with TBARS both in stressed and unstressed compared with control group. Hence it can be concluded that memory enhancing activity might be related to reduction in AChE and TBARS activity and by elevated GSH, SOD and catalase through decrease in raised plasma corticosterone levels.” As taken from Falls N et al. 2018. Biomed. Pharmacother. 106, 575-583. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29990845

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

“BACKGROUND: NAUSEA AND VOMITING OF PREGNANCY ARE AMONGST THE MOST COMMON COMPLAINTS THAT EFFECTS ON BOTH THE PHYSICAL AND MENTAL CONDITIONS OF THE PREGNANT WOMEN. DUE TO THE INCREASING TENDENCY OF WOMEN TO USE HERBAL MEDICATIONS DURING PREGNANCY, THE EFFECT OF LEMON INHALATION AROMATHERAPY ON NAUSEA AND VOMITING OF PREGNANCY WAS INVESTIGATED IN THIS STUDY. OBJECTIVES: The aim of this study was to determine the effect oflemoninhalation aromatherapy on nausea and vomiting during pregnancy. MATERIALS AND METHODS: This was a randomized clinical trial in which 100 pregnant women with nausea and vomiting who had eligibility criteria were randomly divided into intervention and control groups based on four- and six-random block sampling method.Lemonessentialoiland placebo were given to the intervention and control groups, respectively, to inhale it as soon as they felt nausea. The nausea, vomiting, and retch intensity were investigated 24 hours before and during the four days of treatment by means of PUQE-24 (24-hour Pregnancy Unique Quantification of Emesis). RESULTS: There was a statistically significant difference between the two groups in the mean scores of nausea and vomiting on the second and fourth days ($P = 0.017$ and $P = 0.039$, respectively). The means of nausea and vomiting intensity in the second and fourth days in the intervention group were significantly lower than the control group. In addition, in intragroup comparison with ANOVA with repeated measures, the nausea and vomiting mean in the five intervals, showed a statistically significant difference in each group ($P < 0.001$ and $P = 0.049$, respectively). CONCLUSIONS:Lemonscent can be effective in reducing nausea and vomiting of pregnancy.” As taken from Yavari Kia P et al. 2014. Iran. Red Crescent Med. J. 16(3), e14360. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24829772

"Diabetic patients wound healing is slower than the healthy individuals. Three citrus peel extracts; Lemon (Citrus limon), Grapes fruits (Citrus paradise) and Orange (Citrus sinensis) promote wound healing in experimental animals. This study investigated the effect of oral treatment with citrus peel extracts on wound repair of the skin of diabetic rats. The extracts were estimated for vitamin C and total carotenoid contents prior to animal study. Diabetes mellitus was induced in rats by

intraperitoneal injection of a single dose of streptozotocin (STZ, 75 mg kg(-1) b.wt.). One week after diabetes induction, full thickness excision wounds were made in hyperglycemic rats and were divided groups, each containing 6 rats. The different test group animals were treated with different citrus peel extract orally at the dose of 400 mg kg(-1) body weight daily for 12 days. The blood glucose, body weight and rate of wound closure of each rat were measured every 3rd day during the experimental period. At the end of experiment, granular tissues of wounds were removed and estimated for hydroxylproline and total protein content. The results showed significant reduction in blood glucose and time to wound closure. Tissue growth and collagen synthesis were significantly higher as determined by total protein and hydroxyl proline content. From our experimental data, we propose that oral administration of citrus peel extracts has a therapeutic potential in the treatment of chronic wounds in diabetes." As taken from Ahmad M et al. 2013. Pak. J. Biol. Sci. 16(20), 1086-94. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24506007

"Alcoholic liver diseases and virus-induced hepatic dysfunctions are prevalent in western countries. Evidence also suggests that hyperglycemia and insulin resistance are key players in the development of hepatic diseases and their complications. Since the comorbid diseases like obesity, diabetes and vascular dysfunctions primarily affect liver, the modern therapies against other hepatic dysfunctions are becoming a major challenge to treat. In addition to these, polypharmacy and adverse drug reactions (ADRs) are further aggravating the phenomenon. Production of interleukins (IL) 1β, tumor necrosis factors (TNF) α, nuclear factor (NF) κB, activator protein (AP) 1, macrophage inflammatory protein (MIP), toll-like receptor (TLR) 4, and several other harmful cytokines are often evaluated for clinical significance in hepatic complications as recommended by much evidence. On the other hand, transforming growth factors (TGF) β, matrix metalloproteinases, and extracellular matrix- (ECM-) mediated hepatic fibrosis have been identified as major targets. However, modern medicines rely not only on synthetic compounds but also on herbal sources. Traditional therapies are gradually being acknowledged due to having fewer ADRs and other complications. Citrus fruits are generally seen all over the world and offer a great value as seasonal fruit. Several important biologically active components such as polyphenols, flavonols, carbohydrates, amino acids, and oils have been isolated from this family. Evidence suggests that polyphenol-based therapies have already proved their high potency against the production of inflammatory cytokines and profibrogenic factors. Along with the prevention of oxidative stress, these molecules hinder the generation of free radicals. Furthermore, polyphenols induce several defensive genes such as Nrf2, AMPK, superoxide dismutase, catalase, heme oxygenase (HO), Sirt1, and other important functional proteins to serve mitochondrial biogenesis. Therefore, this review will try to establish some molecular theories between citrus polyphenols and liver dysfunctions." As taken from Mohib M et al. 2018. J. Diet. Suppl. 15(2), 223-250. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/28641051

### *7. Addiction*

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

### *8. Burnt ingredient toxicity*

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

Tobacco smoke condensates from cigarettes containing Lemon oil, extract (and other extractables) 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 Lemon oil, extract (and other extractables). Table below provides tested level(s) and specific endpoint(s).

| Endpoint | Tested level (ppm) | Reference |
|---|---|---|
| Smoke chemistry | 2 (oil)<br><1 (oil terpenes, No CAS) | Carmines 2002 &<br>Rustemeier et al., 2002 |
| | 455 | JTI KB Study Report(s) |
| | 261 | Roemer et al., 2014 |
| In vitro genotoxicity | 2 (oil)<br><1 (oil terpenes, No CAS) | Carmines 2002 &<br>Roemer et al., 2002 |
| | 455 | JTI KB Study Report(s) |
| | 30,8 | fGLH Study Report (2010) |
| | 261 | Roemer et al., 2014 |
| In vitro cytotoxicity | 2 (oil)<br><1 (oil terpenes, No CAS) | Carmines 2002 &<br>Röemer et al., 2002 |
| | 455 | JTI KB Study Report(s) |
| | 30,8 | fGLH Study Report (2010) |
| | 261 | Roemer et al., 2014 |
| Inhalation study | 2 (oil)<br><1 (oil terpenes, No CAS) | Carmines 2002 &<br>Vanscheeuwijck et al., 2002 |
| | 455 | JTI KB Study Report(s) |
| | 261 | Schramke et al., 2014 |
| Skin painting | 455 | JTI KB Study Report(s) |
| In vivo genotoxicity | 261 | Schramke et al., 2014 |

## *9. Heated/vapor emissions toxicity*

Aerosol from an electronic nicotine delivery system (ENDS) that creates a vapor by heating an e-liquid containing Lemon oil (and other extractables) was tested in a battery of in vitro 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) 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 responses upon exposure. The table below provides the highest tested level(s) and specific endpoint(s):

| Endpoint | Tested level (ppm) | Reference |
|---|---|---|
| Aerosol chemistry | 500 | Labstat International Inc. (2021) |
| In vitro genotoxicity | 500 | Labstat International Inc. (2022) |
| In vitro cytotoxicity | 500 | Labstat International Inc. (2022) |

## *10. Ecotoxicity*

### *10.1. Environmental fate*

EPISuite provides the following data:

| | |
|---|---|
| **Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:**Bond Method : | 3.80E-001 atm-m3/mole (3.85E+004 Pa-m3/mole) |
| Group Method: | Incomplete |
| Exper Database: | 3.19E-02 atm-m3/mole (3.23E+003 Pa-m3/mole) |
| Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]: | HLC: 5.674E-002 atm-m3/mole (5.749E+003 Pa-m3/mole) |

| | |
|---|---|
| | VP: 1.45 mm Hg (source: MPBPVP)<br>WS: 4.58 mg/L (source: WSKOWWIN) |

| | |
|---|---|
| **Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:**Log Kow used: | 4.38 (exp database) |
| Log Kaw used: | 0.115 (exp database) |
| Log Koa (KOAWIN v1.10 estimate): | 4.265 |
| Log Koa (experimental database): | None |

| | |
|---|---|
| **Probability of Rapid Biodegradation (BIOWIN v4.10):** Biowin1 (Linear Model): | 0.6827 |
| Biowin2 (Non-Linear Model) : | 0.7454 |
| Biowin3 (Ultimate Survey Model): | 2.8981 (weeks) |
| Biowin4 (Primary Survey Model) : | 3.6512 (days-weeks) |
| Biowin5 (MITI Linear Model) : | 0.3978 |
| Biowin6 (MITI Non-Linear Model): | 0.3312 |
| Biowin7 (Anaerobic Linear Model): | 0.1358 |
| Ready Biodegradability Prediction: | NO |

| | |
|---|---|
| **Hydrocarbon Biodegradation (BioHCwin v1.01):**LOG BioHC Half-Life (days): | 0.5696 |
| BioHC Half-Life (days): | 3.7120 |

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

| | |
|---|---|
| Vapor pressure (liquid/subcooled): | 192 Pa (1.44 mm Hg) |
| Log Koa (Koawin est): | 4.265 |
| Kp (particle/gas partition coef. (m3/ug)):<br>Mackay model:<br>Octanol/air (Koa) model: | 1.56E-008<br>4.52E-009 |

Fraction sorbed to airborne particulates (phi):

| | |
|---|---|
| Junge-Pankow model: | 5.64E-007 |
| Mackay model: | 1.25E-006 |
| Octanol/air (Koa) model: | 3.61E-007 |

| | |
|---|---|
| **Atmospheric Oxidation (25 deg C) [AopWin v1.92]:**Hydroxyl Radicals Reaction: OVERALL OH Rate Constant = | 145.2177 E-12 cm3/molecule-sec |
| Half-Life = | 0.074 Days (12-hr day; 1.5E6 OH/cm3) |
| Half-Life = | 0.884 Hrs |

Ozone Reaction:

| | |
|---|---|
| OVERALL Ozone Rate Constant = | 89.924995 E-17 cm3/molecule-sec |
| Half-Life = | 0.013 Days (at 7E11 mol/cm3) |
| Half-Life = | 18.351 Min |
| Fraction sorbed to airborne particulates (phi): 9.07E-007 (Junge-Pankow, Mackay avg) 3.61E-007 (Koa method)Note: the sorbed fraction may be resistant to atmospheric oxidation | |

| | |
|---|---|
| **Soil Adsorption Coefficient (KOCWIN v2.00):**Koc : | 559.9 L/kg (MCI method) |
| Log Koc: | 2.748 (MCI method) |
| Koc : | 6324 L/kg (Kow method) |
| Log Koc: | 3.801 (Kow method) |

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

| | |
|---|---|
| **Volatilization from Water:** Henry LC: 0.0319 atm-m3/mole (Henry experimental database) Half-Life from Model River: | 1.212 hours |
| Half-Life from Model Lake: | 111.1 hours (4.629 days) |

| | |
|---|---|
| **Removal In Wastewater Treatment:** Total removal: | 94.42 percent |
| Total biodegradation: | 0.14 percent |
| Total sludge adsorption: | 31.45 percent |
| Total to Air: | 62.83 percent |

(using 10000 hr Bio P,A,S)

| **Level III Fugacity Model:** | Mass Amount(percent) | Half-Life(hr) | Emissions(kg/hr) |
|---|---|---|---|
| Air | 0.394 | 0.334 | 1000 |
| Water | 64.4 | 360 | 1000 |
| Soil | 33.8 | 720 | 1000 |
| Sediment | 1.39 | 3.24e+003 | 0 |

Persistence Time: 95.9 hr

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that oils, lemon are of uncertain persistence in the environment.

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

### *10.2. Aquatic toxicity*

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that oils, lemon are not inherently toxic to aquatic organisms and are of low ecotoxicological concern.

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

“Tests on lemon peel oil extract as a mosquito larvicide were carried out. The oil was found to be toxic on larvae, pupae and eggs of Culex quinquefasciatus. The oil also fulfilled other required specifications like suitable specific gravity, spreading pressure and viscosity. It was also toxic at a wide pH range, stable to heat and light in terms of chemical change which could alter larvicidal action. However, it was volatile and did not form a permanent film on water surfaces for long periods. This affected the larvicidal action” (Mwaiko & Savaeli, 1994).

ECOSAR version 1.11 reports the following aquatic toxicity data for CAS RN 8008-56-8:

Values used to Generate ECOSAR Profile:

Log Kow: 4.827 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 13.8 (mg/L, PhysProp DB exp value)

ECOSAR v1.11 Class-specific Estimations:
Neutral Organics

| ECOSAR Class | Organism | Duration | End Pt | Predicted mg/L (ppm) |
|---|---|---|---|---|
| Neutral Organics : | Fish | 96-hr | LC50 | 0.323 |
| Neutral Organics : | Daphnid | 48-hr | LC50 | 0.238 |
| Neutral Organics : | Green Algae | 96-hr | EC50 | 0.522 |
| Neutral Organics : | Fish | | ChV | 0.043 |
| Neutral Organics : | Daphnid | | ChV | 0.048 |
| Neutral Organics : | Green Algae | | ChV | 0.245 |
| Neutral Organics : | Fish (SW) | 96-hr | LC50 | 0.414 |
| Neutral Organics : | Mysid | 96-hr | LC50 | 0.045 |

| | | | | |
|---|---|---|---|---|
| Neutral Organics : | Fish (SW) | | ChV | 0.253 |
| Neutral Organics : | Mysid (SW) | | ChV | 0.00169 |

### *10.3. Sediment toxicity*

No data available to us at this time.

### *10.4. Terrestrial toxicity*

“The vine mealybug, Planococcus ficus (Signoret) (Hemiptera: Pseudococcidae), is a pest in grape vine growing areas worldwide. The essential oils from the following aromatic plants were tested for their insecticidal activity against P. ficus: peppermint, Mentha piperita L. (Lamiales: Lamiaceae), thyme-leaved savory, Satureja thymbra L., lavender, Lavandula angustifolia Mill, and basil, Ocimum basilicum L. Essential oils from peels of the following fruits were also tested: lemon, Citrus limon L. (Sapindales: Rutaceae), and orange, C. sinensis L. The reference product was paraffin oil. Bioassays were conducted in the laboratory by using spray applications on grape leaves bearing clusters of P. ficus of one size class, which mainly represented either 3rd instar nymphs or pre-ovipositing adult females. The LC50 values for each essential oil varied depending on the P. ficus life stage but did not significantly differ between 3(rd) instar nymphs and adult females. The LC50 values of the citrus, peppermint, and thyme-leaved savory essential oils ranged from 2.7 to 8.1 mg/mL, and the LC50 values of lavender and basil oil ranged from 19.8 to 22.5 and 44.1 to 46.8mg/mL, respectively. The essential oils from citrus, peppermint and thymeleaved savory were more or equally toxic compared to the reference product, whereas the lavender and basil essential oils were less toxic than the paraffin oil. No phytotoxic symptoms were observed on grape leaves treated with the citrus essential oils, and low phytotoxicity was caused by the essential oils of lavender, thyme-leaved savory, and mint, whereas the highest phytotoxicity was observed when basil oil was used.” As taken from Karamaouna F et al. 2013. J. Insect. Sci. 13(142), 1-3. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24766523

“Peel oils of lemon, grapefruit and navel orange were tested for insecticidal activities against larvae and adults of Culex pipiens and Musca domestica. Lemon peel oil was the most effective against larvae and adults of C. pipiens. Grapefruit peel oil was more toxic to adults of M. domestica while lemon oil, was more toxic Musca larvae. On the other hand, the orange peel oil was the least effective against larvae and adults of both species. The toxicity of oils applied to larval stages was extended to pupal and adult stages. C. pipiens adults appeared with paralyzed legs, while M domestica adults appeared normal. The weights of pupae treated as larvae were generally less than that of the control. All oils produced deleterious effects on fecundity of survivors of sublethal doses. The effect was obviously recorded in treated adults. Treatment of Culex & Musca with oils caused serious latent effect” (Shalaby et al. 1998).

“The larviciddal action of three ethanol extracts of peel oils of lemon, grapefruit and navel orange were tested against the early 4th instar larvae of Culex pipiens and the resulting pupae. The LC50 were 18.5, 20.3 and 26.5 and the slope functions were 2.9, 2.9 and 3.9 respectively. The action of the lemon extract extended to the pupae which resulted from larvae exposed to sublethal dose. Some of the pupae were unable to escape from the larval exuviae” (Al Dakhil & Morsy, 1999).

Record for oils, lemon (CAS RN 8008-56-8):

| Spec.Sci. Name<br>Spec. Common. Name | Resp. Site<br>Exp. Dur. (Days) | Media Type<br>Test Loc. | Exp. Type<br>Chem. Anal. | Dose # Res.<br>Sample Unit | Endpoint<br>BAF/BCF | Effect<br>Effect Meas. | Signif.<br>Sig. Level | Dose<br>Dose Stat. Meth. |
|---|---|---|---|---|---|---|---|---|
| Trichoderma harzianum<br>Fungus | 1 | CUL<br>LAB | CM<br>U | | LOEL | MOR<br>MORT | SIG<br><0.05 | F (10.0-15.0)<br>ul/ml |
| Trichoderma harzianum<br>Fungus | 1 | CUL<br>LAB | CM<br>U | | LOEL | MOR<br>MORT | SIG<br><0.05 | F (10.0-15.0)<br>ul/ml |
| Trichoderma harzianum<br>Fungus | 1 | CUL<br>LAB | CM<br>U | | LOEL | MOR<br>MORT | SIG<br><0.05 | F (10.0-15.0)<br>ul/ml |
| Verticillium fungicola<br>Fungus | 1 | CUL<br>LAB | CM<br>U | | LOEL | MOR<br>MORT | SIG<br><0.05 | F (10.0-15.0)<br>ul/ml |
| Trichoderma harzianum<br>Fungus | MYC<br>21 | CUL<br>LAB | CM<br>U | | LOEL | POP<br>ABND | SIG<br><0.05 | F (25.0-35.0)<br>ul/ml |
| Trichoderma harzianum<br>Fungus | MYC<br>21 | CUL<br>LAB | CM<br>U | | LOEL | POP<br>ABND | SIG<br><0.05 | F (25.0-35.0)<br>ul/ml |
| Trichoderma harzianum<br>Fungus | MYC<br>21 | CUL<br>LAB | CM<br>U | | LOEL | POP<br>ABND | SIG<br><0.05 | F (25.0-35.0)<br>ul/ml |
| Verticillium fungicola<br>Fungus | MYC<br>21 | CUL<br>LAB | CM<br>U | | LOEL | POP<br>ABND | SIG<br><0.05 | F (25.0-35.0)<br>ul/ml |
| Trichoderma harzianum<br>Fungus | MYC<br>21 | FLT<br>LAB | DA<br>U | | LOEL | POP<br>ABND | SIG<br><0.05 | F (> 27-< 30)<br>ul/eu |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Trichoderma harzianum<br>Fungus | MYC<br>21 | FLT<br>LAB | DA<br>U | | LOEL | POP<br>ABND | SIG<br><0.05 | F (> 27-< 30) ul/eu |
| Trichoderma harzianum<br>Fungus | MYC<br>21 | FLT<br>LAB | DA<br>U | | LOEL | POP<br>ABND | SIG<br><0.05 | F (> 27-< 30) ul/eu |
| Verticillium fungicola<br>Fungus | MYC<br>21 | FLT<br>LAB | DA<br>U | | LOEL | POP<br>ABND | SIG<br><0.05 | F (> 27-< 30) ul/eu |
| Trichoderma harzianum<br>Fungus | MYC<br>1 | CUL<br>LAB | CM<br>U | | LOEL | POP<br>ABND | SIG<br><0.05 | F (10.0-15.0) ul/ml |
| Trichoderma harzianum<br>Fungus | MYC<br>1 | CUL<br>LAB | CM<br>U | | LOEL | POP<br>ABND | SIG<br><0.05 | F (10.0-15.0) ul/ml |
| Trichoderma harzianum<br>Fungus | MYC<br>1 | CUL<br>LAB | CM<br>U | | LOEL | POP<br>ABND | SIG<br><0.05 | F (10.0-15.0) ul/ml |
| Verticillium fungicola<br>Fungus | MYC<br>1 | CUL<br>LAB | CM<br>U | | LOEL | POP<br>ABND | SIG<br><0.05 | F (10.0-15.0) ul/ml |

As taken from the EPA ECOTOX database.

ECOSAR version 1.11 reports the following terrestrial toxicity data for CAS RN 8008-56-8:

Values used to Generate ECOSAR Profile:

Log Kow: 4.827 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 13.8 (mg/L, PhysProp DB exp value)

ECOSAR v1.11 Class-specific Estimations:
Neutral Organics

| ECOSAR Class | Organism | Duration | End Pt | Predicted mg/L (ppm) |
|---|---|---|---|---|
| Neutral Organics : | Earthworm | 14-day | LC50 | 120.584 * |

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.

“The toxicity of essential oils from the citrus peel has been proposed as the major resistance mechanism offered by citrus to fruit fly infestation. We evaluated the insecticidal activity of the ether extracts from the lemon (Citrus limon [L.] Burm.) and grapefruit (C. paradisi Macfadyen) peel as well as from limonene and citral against Anastrepha fraterculus (Wiedemann) and Ceratitis capitata (Wiedemann) immature stages. We also evaluated the toxicity of the extracts at two ripening stages. Extracts proved toxic to A. fraterculus egg and larvae. The lemon and grapefruit extracts showed the same toxicity in both fruit fly species. For A. fraterculus eggs, citral was more toxic than limonene; for larvae, they showed equal toxicity. Anastrepha fraterculus eggs were more sensitive than C. capitata eggs. In conclusion, we provide evidence of chemical resistance mechanisms that could account for the nonhost condition of lemon for A. fraterculus.” As taken from Ruiz MJ et al. 2014. J. Agric. Food Chem. 62(41), 10084-10091. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/25237738

“The fumigant, contact, and repellent activities of four essential oils extracted from Citrus limonum (Sapindales: Rutaceae), Litsea cubeba (Laurales: Lauraceae), Cinnamomum cassia, and Allium sativum L. (Asparagales: Alliaceae) against 6th instars and adults of the darkling beetle, Alphitobius diaperinus (Panzer) (Coleoptera: Tenebrionidae), one of the main pests of materials and products of Juncus effuses L. (Poales: Juncaceae) during the storage period, were assayed, and chemical ingredients were analyzed with gas chromatography-mass spectrometry in this study. While the major ingredients found in C. limonum and C. cassia were limonene and (E)-cinnamaldehyde, the main constituents of L. cubea were D-limonene, (E)-3,7-dimethyl-,2,6-octadienal, (Z)-3,7-dimethyl,2 ,6-octadienal, and diallyl disulphide (18.20%), while the main constituents of and A. sativum were di-2-propenyl trisulfide and di-2-propenyl tetrasulfide. The fumigation activities of A. sativum and C. limonum on A. diaperinus adults were better than those of the other two essential oils. The toxicities of A. sativum and C. limonum were almost equitoxic at 96 hr after treatment. Essential oils from Allium sativum and L. cubeba also showed good contact activities from 24 hr to 48 hr, and toxicities were almost equitoxic 48 hr posttreatment. The repellent activities of A. sativum and L. cubeba oils on 6th instars were also observed, showing repellence indexes of 90.4% and 88.9% at 12 hr after treatment, respectively. The effects of A. sativum on AChE activity of 6th instars of A. diaperinus were strongest compared to the other essential oils, followed by C. limonum, L. cubeba, and C. cassia. These results suggest that the essential oils of C. limonum and A. sativum could serve as effective control agents of A. diaperinus.” As taken from Wang X et al. 2014. J. Insect Sci. 14, 75. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/25373222

“Plant essential oils have been suggested as a promising alternative to the established mosquito repellent DEET (N,N-diethyl-meta-toluamide). Searching for an assay with generally available equipment, we designed a new audiovisual assay of repellent activity against mosquitoes "Singing in the Tube," testing single mosquitoes in Drosophila cultivation tubes. Statistics with regression analysis should compensate for limitations of simple hardware. The assay was established with female Culex pipiens mosquitoes in 60 experiments, 120-h audio recording, and 2580 estimations of the distance between mosquito sitting position and the chemical. Correlations between parameters of sitting position, flight activity pattern, and flight tone spectrum were analyzed. Regression analysis of psycho-acoustic data of audio files (dB[A]) used a squared and modified sinus function determining wing beat frequency WBF ± SD (357 ± 47 Hz). Application of logistic regression defined the repelling velocity constant. The repelling velocity constant showed a decreasing order of efficiency of plant essential oils: rosemary (Rosmarinus officinalis), eucalyptus (Eucalyptus globulus), lavender (Lavandula angustifolia), citronella (Cymbopogon nardus), tea tree (Melaleuca alternifolia), clove (Syzygium aromaticum), lemon (Citrus limon), patchouli (Pogostemon

cablin), DEET, cedar wood (Cedrus atlantica). In conclusion, we suggest (1) disease vector control (e.g., impregnation of bed nets) by eight plant essential oils with repelling velocity superior to DEET, (2) simple mosquito repellency testing in Drosophila cultivation tubes, (3) automated approaches and room surveillance by generally available audio equipment (dB[A]: ISO standard 226), and (4) quantification of repellent activity by parameters of the audiovisual assay defined by correlation and regression analyses." As taken from Adams TF et al. 2016. Parasitol. Res. 115(1), 225-39. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26412058

"The effect of lemon oil (Citrus limon) on Sarcoptes scabiei var. cuniculi was evaluated in vitro and in vivo. The mite samples were collected from naturally infected rabbits. The lemon oil was prepared in six concentrations by dilution with distilled water (2.5, 5, 10, 20, 50, and 100 %). In vitro application was done in five replicates for each concentration in petri dishes in the laboratory. The treated mites were observed at 1, 12, and 24 h post application (PA) for lemon oil effect. In addition, oxidative stress profile was evaluated for the treated mite. Dependent on in vitro results, 20 % lemon oil was used in vivo trial. Twenty-four naturally infected rabbits were divided into three groups of eight: 20 % lemon oil, deltamethrin, and untreated control. The infected parts of rabbits were treated topically once a week for four successive weeks. In vitro application results showed that lemon oil 10 and 20 % diluted in water caused mortality to 100 % of mites after 24 h PA. The oxidative stress profile revealed that mites treated with 20 % lemon oil had significantly ($P < 0.05$) higher hydrogen peroxide and malondialdehyde when compared with mites treated with deltamethrin or distilled water. In vivo application of 20 % lemon oil on naturally infected rabbits showed complete recovery from clinical signs, absence of mite in microscopic examination from the second week of treatment. In addition, productive performance was significantly better than infected untreated group. Also, the treated tissue showed stoppage of scale formation and hair growth faster than deltamethrin-treated rabbits. Consequently, lemon oil has remarkable miticidal activity in vitro and in vivo applications." As taken from Aboelhadid SM et al. 2016. Parisitol. Res. 115(8), 3013-20. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27098160

### *10.5. All other relevant types of ecotoxicity*

EPISuite provides the following data:

**Bioaccumulation Estimates (BCFBAF v3.01):**

| | |
|---|---|
| Log BCF from regression-based method: | 2.557 (BCF = 360.5 L/kg wet-wt) |
| Log Biotransformation Half-life (HL): | 0.5478 days (HL = 3.531 days) |
| Log BCF Arnot-Gobas method (upper trophic): | 2.963 (BCF = 917.8) |
| Log BAF Arnot-Gobas method (upper trophic): | 2.969 (BAF = 931.1) |
| log Kow used: | 4.38 (expkow database) |

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that oils, lemon are of uncertain bioaccumulative potential in the environment.

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

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### *13. Last audited*

May 2022

# Lemon Oil
# [CASRN 8008-56-8]

# Lime Oil
# [CASRN 8008-26-2]

Prepared October 2000

## SUMMARY

Lemon oil and lime oil are added as fragrances to a number of perfumed cosmetic products. Many of these products are intended for use on sun-exposed skin. Clinical and experimental studies have found that both lemon oil and lime oil are phototoxic. Because of their phototoxicity, the International Fragrance Association has recommended safe use levels for "leave on products" applied to sun-exposed skin. These safe use levels are intended to avoid short-term phototoxicity. However, long-term effects have not been addressed.

The phototoxicity elicited by lemon oil and lime oil has been associated with naturally occurring furocoumarins. Two of these furocoumarins, 5-methoxypsoralen and oxypeucedanin, have been identified as the probable phototoxins in both lemon oil and lime oil. The levels of these furocoumarins have been found to vary significantly with growing conditions.

While the photogenotoxicity of 5-methoxypsoralen is well established, the photogenotoxicity of oxypeucedanin is unknown. Bacterial mutagenesis studies indicated that oxypeucedanin is not mutagenic, however photogenotoxicity studies of oxypeucedanin are not available. Additionally, the mutagenicity and photomutagenicity of mixtures of coumarins and furocoumarins in lemon oil and lime oil have not been assessed.

# TABLE OF CONTENTS

**SUMMARY …… i**

**1.0 BASIS FOR NOMINATION…… 1**

**2.0 INTRODUCTION……. 1**

**2.1 Chemical Identification…… 1**
**2.2 Physical-Chemical Properties…….. 2**

**3.0 PRODUCTION PROCESSES……. 2**

**4.0 REGULATORY STATUS…… 2**

**5.0 OCCURRENCE IN COSMETICS…….. 2**

**6.0 TOXICOLOGICAL ISSUES…….. 3**

**6.1 Phototoxicity of Lemon Oil and Lime Oil…….. 3**
**6.2 Coumarins and Furocoumarins Occurring in Lemon Oil and Lime Oil……. 5**

**7.0 REQUESTED STUDIES……. 7**

**8.0 REFERENCES…… 7**

## 1.0 BASIS FOR NOMINATION

Lemon oil and lime oil were selected by the Center for Food Safety and Applied Nutrition for photogenotoxicity and photocarcinogenesis studies since these phototoxic oils contain furocoumarins. Furocoumarins such as 5-methoxypsoralen have been shown to be photocarcinogenic and are present in lemon and lime oils.

## 2.0 INTRODUCTION

### 2.1 Chemical Identification

| | **Lemon Oil** | **Lime Oil** |
|---|---|---|
| CASRN: | 8008-56-8 | 8008-26-2 |
| RTECS: | OG8300000 | RI7270000 |

The following synonyms are used for lemon oil:

- *Citrus Medica Limonum* Oil [International Nomenclature of Cosmetic Ingredients (INCI) name, CTFA, 1999]
- Oil of Lemon (Budavari, 1996)

The following synonyms are used for lime oil:

- *Citrus Aurantifolia* Oil (INCI name, CTFA, 1999)
- Oil of Lime (RTECS)

### 2.2 Physical-Chemical Properties of Lemon Oil and Lime Oil (Table 1)

| **Property** | **Lemon Oil** | **Lime Oil** |
|---|---|---|
| Color[1] | Pale to deep yellow or greenish yellow | Colorless to greenish yellow |
| Odor[1] | Fresh citrus, intense | Fresh citrus, intense |
| Specific Gravity[1] | 0.849-0.855 | 0.855-0.863 |
| Refractive Index[1] | 1.474-1.467 | 1.4477-1.4745 |
| Optical Rotation[1] | +57 to +65.6 | +34 to +47 |
| Stability[2] | Air/light sensitive | Air/light sensitive |
| Solubility | | |
| Water[1] | insol. | insol. |
| Ethanol[1] | sol. | sol. |
| Propylene glycol[1] | sol. | sol. |

[1]Secondini, 1990.
[2] Poucher, 1991.

## 3.0 PRODUCTION PROCESSES

Several processes are used to produce lemon oil and lime oil. Expressed lemon oil and lime oil are produced by pressing the outer rind of the ripe fruits by sponge-press (*i.e.*, by hand) or machines (Poucher , 1991). These oils may be produced more economically using an integrated juice-oil procedure such as the Brown Oil Extractor (Swains and Swains, 1988), where citrus fruit is partially submerged in water and abraded by metal discs. The oil is separated from the juice as a water-emulsion, and further separated using centrifugation to obtain the oil. The yield of expressed oil is approximately 4% for lemons (somewhat less for limes) based on the weight of whole fruit (Burdock, 1995). Lemon and lime oils are also produced by distillation of expressed oils or direct distillation of fruit (Swaine and Swaine, 1988; Burdock, 1995). Lemon oil and lime oil are distilled (rectified) for removal of terpenes in order to improve solubility and permit use for flavoring carbonated beverages (Burdock, 1995). Lemon oil and lime oil can also be steam distilled to remove nonvolatile furocoumarins and are subsequently marketed as "psoralen free" (LOLI Global Industries). The types of lemon oil and lime oil added to cosmetics are not regulated by law, nor limited by recommendations of appropriate trade organizations.

## 4.0 REGULATORY STATUS

Lemon oil and lime oil are approved as GRAS for food use (CFR, 2000). The Food, Drug and Cosmetic Act does not authorize the Food and Drug Administration to require pre-market approval for ingredients added to cosmetics.

## 5.0 OCCURRENCE IN COSMETICS

Data available from FDA's Voluntary Cosmetics Registration Program (VCRP), compiled in accordance with Title 21 part 270.4 (d)(1) of the Code of Federal Regulations (Code of Federal Regulations, 2000b), indicate that lemon oil is found in 98 cosmetic formulations and lime oil is in 14 formulations.

Both lemon and lime oil are primarily used as fragrances in perfumed cosmetics (Cosmetic, Toiletry, and Fragrance Association, 1999). The International Fragrance Association (IFRA) has made specific recommendations for use of lemon and lime oil in cosmetic formulations (IFRA, 1992). IFRA recommends that *"for applications on areas of skin exposed to sunshine, excluding bath preparations, soaps and other products which are washed off the skin, lemon oil cold pressed should not be used such that the level in the consumer products exceeds 2%."* Similar recommendations are made for lime oil except that the level in consumer products is recommended not to exceed 0.7% (IFRA, 1992). IFRA makes no recommendation for removal of nonvolatile photosensitizing components by distillation for either lemon or lime oil.

Typical concentrations in final products are given in Table 2.

**Table 2. Typical Concentrations of Citrus Oils in Finished Products**
(Opdyke, 1974)

| Ingredient | Soap | Detergent | Cream/Lotion | Perfume |
|---|---|---|---|---|
| | Usual/Maximum | Usual/Maximum | Usual/Maximum | Usual/Maximum |
| Lemon Oil (Expressed) | 0.05/0.4[1] | 0.005/0.04 | 0.03/0.15 | 0.5/1.0 |
| Lime Oil (Expressed) | 0.05/0.25 | 0.005/0.025 | 0.03/0.1 | 0.5/1.5 |
| Lime Oil (Distilled) | 0.05/0.25 | 0.005/0.025 | 0.03/0.1 | 0.4/1.5 |

[1] w/w %

## 6.0 TOXICOLOGICAL ISSUES

### 6.1 Phototoxicity of Lemon Oil and Lime Oil.

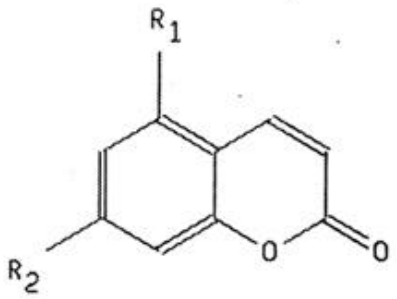
The phototoxicity of lemon oil and lime oil has been demonstrated in both experimental models and humans. Naganuma *et al.*, 1985, studied the phototoxicity of lemon oil obtained from different geographic locations. Lemon oil was diluted in ethanol (100% lemon oil, 50% lemon oil, and 20% lemon oil) and applied to backs of shaved, albino guinea pigs. Animals were then exposed to UVA radiation (320-400 nm, 13 J/cm$^2$). Erythema was evaluated at 24, 48 and 72 hr after irradiation. Undiluted lemon oil and 50% lemon oil elicited phototoxicity for most of the samples tested. Lemon oil from Australia and Brazil elicited a weaker phototoxic than lemon oil from the Ivory Coast, Sicily, California, or Argentina. In an effort to identify the phototoxic components in lemon oil, these investigators fractionated samples by solvent extraction and subsequent phototoxicity testing of isolated components. Two phototoxic components were identified: oxypeucedanin and 5-methoxypsoralen (see Figure 1).

Forbes *et al.*, 1977, have investigated the phototoxicity of a large number of fragrance raw materials including lemon oil and lime oil. Fragrance raw materials were tested using humans, pigs and albino, hairless mice. Several irradiation sources were used including sunlight, a solar simulator and a UVA radiation source (black light). Expressed lime oil was phototoxic in all three species under all three radiation sources. Distilled lime oil usually elicited no phototoxic response in any species. Two samples of distilled lime oil (derived from expressed lime oil), however, were found to be phototoxic. Lemon oil from Greece, Italy and from Florida (Persian limes) was reported to be phototoxic.

**Figure 1. Structures of Major Coumarins and Furocoumarins in Lemon Oil and Lime Oil.**

$R_1 = OCH_3$; $R_2 = OCH_3$
5,7-Dimethoxycoumarin (Citroptene, Limettin)

$R_1 = OCH_2CH=C(CH_3)CH_2CH_2CH=C(CH_3)_2$;
$R_2 = OCH_3$
Geranoxy-7-methoxycoumarin

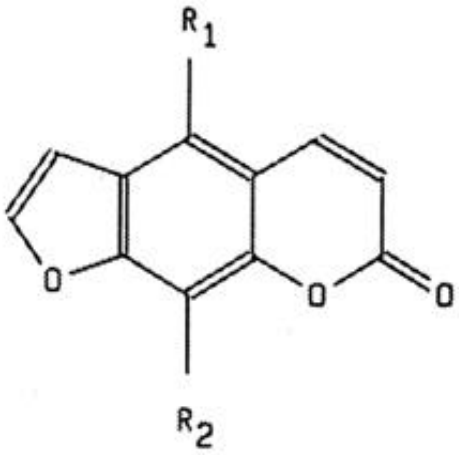



$R_1 = H$; $R_2 = H$
Psoralen

$R_1 = H$; $R_2 = OCH_3$
8-Methoxypsoralen (Xanthotoxin)

$R_1 = OCH_3$; $R_2 = H$
5-Methoxypsoralen (Bergapten)

$R_1 = OCH_2CH=C(CH_3)CH_2CH_2CH=C(CH_3)_2$;
$R_2 = H$
5-Geranoxypsoralen (Bergamottin)




$R_1 = OCH_3$; $R_2 = OCH_3$
5,7-Dimethoxypsoralen (Isopimpinellin)

$R_1 = OCH_2CH(O)C(CH_3)_2$ (epoxide); $R_2 = H$
5-(Isoamyloxy-2,3-epoxide)psoralen (Oxypeucedanin)

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## 6.2 Coumarins and Furocoumarins Occurring in Lemon Oil and Lime Oil

Investigators have examined the level of coumarins and furocoumarins in lemon oil and lime oil (Table 3). A large body of evidence indicates that these secondary metabolites are photo-activated phytoalexins, produced to protect plants from bacterial, fungal, parasitic, and insectile infestations (Downum, 1992; Heitz, 1987). Consistent with their protective function, levels of coumarins and furocoumarins vary widely. Naganuma *et al.*, 1985, found that levels of oxypeudedanin doubled in lemon oil derived from the same growing area in Australia between 1979 and 1980. The levels of oxypeudedanin in lemon oil from the Ivory Coast, Sicily and California diminished nearly three-fold in the same time period.

**Table 3. Levels of Major Coumarins and Furocoumarins in Lemon Oil and Lime Oil.**

| Compound | Photosensitizing Activity | % in Lemon Oil [1] | % in Lime Oil |
|---|---|---|---|
| 5-Geranoxypsoralen | 0 [1] | 0.0387 | 2.2-2.5 [3] |
| 5-Geranoxy-7-Methoxycoumrin | 0 [1] | 0.0603 | 2.2-5.2 [3] |
| 5-Geranoxy-8-methoxypsoralen | 0 [1] | not analyzed | 0.945 [4] |
| 5,7-Dimethoxycoumarin | 0 [2] | 0.0295 | 0.464 [4] |
| 5,8-Dimethoxypsoralen | 0 [2] | not analyzed | 0.508 [4] |
| Oxypeucedanin | + [1a] | 0.005-0.073 | 0.0025 [4] |
| 5-Methoxypsoralen | ++++ [1] | 0.0001-0.0087 | 0.17-0.33 [3] |

[1] Naganuma *et al.* (1985), phototoxicity tested on both guinea pig and rabbit skin.
[1a] Naganuma *et al.* (1985) found that oxypeucedanin was approximately 4 times weaker than 5- methoxypsoralen in eliciting phototoxicity in the guinea pig.
[2]Musajo and Rodighiero (1962), phototoxicity tested on human skin.
[3] Cieri (1969).
[4] Stanley and Vannier (1967).

The photogenotoxicity and photocarcinogenicity of several linear furocoumarins (psoralens) have been well documented (reviewed by Saffran, 1988). 5-Methoxypsoralen, a component of lemon and lime oil, is photogenototic in both prokaryotic and eukaryotic test systems. In addition, 5-methoxypsoralen has been found to be photocarcinogenic in an experimental model (Zajdela and Bisagni, 1981). Little is known about the photogenotoxicity and photocarcinogenicity of other furocoumarins found in lemon oil and lime oil. The mutagenicity of oxypeucedanin has been assessed in 6 tester strains of *Salmonella typhimurium* by Uwaifo, 1984. Oxypeucedanin was not mutagenic, however, photomutagenicity was not assessed.

## 7.0 REQUESTED STUDIES

Lemon oil and lime oil contain a complex mixture of coumarins and furocoumarins. Two of these compounds, 5-methoxypsoralen and oxypeucedanin, have been identified as phototoxic and implicated in the phototoxicity elicited by these essential oils. While the photogenotoxicity of 5-methoxypsoralen is well established, the photogenotoxicity of oxypeucedanin alone or in mixtures of compounds representative of lemon oil and lime oil has not been assessed. These photogenotoxicity studies are requested. Additional studies on photocarcinogenicity should be conducted if coumarins or furocoumarins are significantly photogenotoxic as individual compounds or in complex mixtures (*e.g.* lemon or lime oil).

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# OPINION OF THE SCIENTIFIC COMMITTEE ON COSMETIC PRODUCTS AND NON-FOOD PRODUCTS INTENDED FOR CONSUMERS

CONCERNING

# AN INITIAL LIST OF PERFUMERY MATERIALS WHICH MUST NOT FORM PART OF COSMETIC PRODUCTS EXCEPT SUBJECT TO THE RESTRICTIONS AND CONDITIONS LAID DOWN

adopted by the SCCNFP during the 18th Plenary meeting
of 25 September 2001

## 1. Terms of Reference

In recent years there has been concern on the safety of fragrance (perfumery) materials. Dermatologists have highlighted the frequency of allergic contact dermatitis from perfumes.

Under current legislation, fragrance materials do not fall under all the requirements of Directive 76/768/EEC on cosmetic products. The 6th Amendment (93/35/EEC) provides for the labelling of ingredients on cosmetic products. However, it is not a requirement to label fragrance constituents on the packaging of cosmetic products, current legislation requires only the word *parfum*.

In response to growing concern over this issue, the Commission was asked for positive actions with respect to legislative measures on fragrance materials.

## 2. Mandate

The SCCNFP has been asked to respond to the following questions :

1. Does the SCCNFP agree to the inclusion of all IFRA restricted materials in the Annex III (List of substances which cosmetic products must not contain except subject to restrictions and conditions laid down)? Are the permitted levels recommended by IFRA suitable for use in the Cosmetics Directive 76/768/EEC ?

2. Does the SCCNFP agree that all materials that IFRA recommend should not be used as fragrance compounds are included in Annex II (List of substances which must not form part of the composition of cosmetic products)?

3. It is proposed that all known fragrance allergens are labelled on cosmetics if used in the products. Does the SCCNFP agree to this proposal? If so :

   - Which chemicals fall under this classification ?
   - Is there a maximum concentration of each chemical permissible without the requirement for labelling ?

4. Restrictions are proposed for the 3 most common fragrance allergens (cinnamic aldehyde, isoeugenol, hydroxycitronellal). Does the SCCNFP agree to restriction on the use of common fragrance allergens (Annex III listing)? If so :

   - Which fragrance materials should be subject to restrictions?
   - What are the conditions for restrictions (maximum concentration, fields of applications, etc) ?

Obviously, in response to each of the questions listed above, a scientific justification will be necessary.

## 3. Strategy of the SCCNFP

The SCCNFP has considered that this mandate can be usefully divided into two sections (Interim position on Fragrance allergy, document n° SCCNF/0202/99 adopted by the SCCNFP during the 8th Plenary meeting of 23 June 99) :

1. Identification of those fragrance ingredients, which are of concern as allergens for the consumer. Recommendations on informing the consumer of the presence of important allergens to permit the consumer with a known fragrance allergy a means to avoid contact with an allergen. An opinion as to whether such identification can be related to concentrations present in a product when elicitation levels are known.

2. An opinion on the adoption of industry prohibited substances into Annex 2 and adoption of industry restricted substances into Annex 3. Considerations as to whether the concentration limits or other restrictions suggested by industry can be supported or need to be changed if there is such inclusion in Annex 3. Whether there are additional substances which should be subject to inclusion in an Annex.

An opinion concerning fragrance allergy was adopted by the SCCNFP during the 10th Plenary meeting of 8 December 1999 (doc. n° SCCNFP/0017/98 final). During its 12th plenary meeting of 3 May 2000, the SCCNFP adopted an opinion on an initial list of perfumery materials which must not form part of fragrance compounds used in cosmetic products (doc. n° SCCNFP/0320/00 final).

The current opinion consists of the adoption of an initial list of perfumery materials to be included in Annex III - List of substances which cosmetic products must not contain except subject to restrictions and conditions laid down - to Directive 76/768/EEC.

The opinion is based on information submitted as ‘monographs’ (synopses) on behalf of industry.

## 4. Opinion

On the basis of the available information and assessment of the cutaneous toxicity of the substances tabulated, it is the recommendation of the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) that these substances may be used as ingredients in cosmetic products only under the conditions and restrictions specified in the attached table.

Additional substances will be discussed for possible inclusion at a later date.

Table 1 : List of perfumery materials which must not form part of cosmetic products except subject to the restrictions and conditions laid down

| N° | Substance name | Restriction and condition |
|---|---|---|
| 1 | *Abies alba oil from cones (Abies Alba Mill.)*<br>CAS n° : 8021-27-0<br><br>*Abies alba oil from needles (Abies Alba Mill.)*<br>CAS n° : 8021-27-0<br><br>*Abies sachalinensis oil*<br>CAS n° : Unkown<br><br>*Fir balsam (Abies balsamea (L.) Mill)*<br>CAS n° : 8021-28-1<br><br>*Fir needle oil (Abies sibirica)*<br>CAS n° : 8021-29-2<br><br>*Fir needle oil, Canadian (Abies balsamea)*<br>CAS n° : 8021-28-1<br><br>*Pine needle, dwarf, oil (Pinus mugo turra var. pumilio (Haenke) Zenari)*<br>CAS n° : 8000-26-8<br><br>*Pine needle oil (Abies spp.)*<br>CAS n° : 8021-29-2<br><br>*Pine scotch oil (Pinus sylvestris L.)*<br>CAS n° : 8023-99-2<br><br>*Pinus nigra oil*<br>CAS n° : 90082-74-9<br><br>*Turpentine gum (Pinus spp.)*<br>CAS n° : 9005-90-7<br><br>*Turpentine oil*<br>CAS n° : 8006-64-2<br><br>*Turpentine oil rectified*<br>CAS n° : 8006-64-2<br><br>*Turpentine, steam distilled (Pinus spp.)*<br>CAS n° : 8006-64-2 | Essential oils and isolates derived from the Pinacea family, including Pinus and Abies genera, should only be used when the level of peroxides is kept to the lowest practicable level, for instance by adding antioxidants at the time of production. Such products should have a peroxide value of less than 10 millimoles peroxide per liter. Based on the published literature mentioning sensitising properties when containing peroxides (Food and Chemical Toxicology 11,1053(1973); 16,843(1978); 16,853(1978). |
| 2 | Acetyl hexamethyl indan<br><br>CAS n° : 15323-35-0 | For applications on areas of skin exposed to sun, excluding bath preparations, soaps and other rinse-off products, limit use to 2%in the finished cosmetic product. Based on the phototoxic potential showing no effects at 10% in human photo-toxicity tests (IFRA guidelines). |

| N° | Substance name | Restriction and condition |
|---|---|---|
| 3 | *Allyl butyrate*<br>CAS n° : 2051-78-7<br><br>*Allyl cinnamate*<br>CAS n° : 1866-31-5<br><br>*Allyl cyclohexaneacetate*<br>CAS n° : 4728-82-9<br><br>*Allyl cyclohexanepropionate*<br>CAS n° : 2705-87-5<br><br>*Allyl heptanoate*<br>CAS n° : 142-19-8<br><br>*Allyl hexanoate*<br>CAS n° : 123-68-2<br><br>*Allyl isovalerate*<br>CAS n° : 2835-39-4<br><br>*Allyl octanoate*<br>CAS n° : 4230-97-1<br><br>*Allyl phenoxyacetate*<br>CAS n° : 7493-74-5<br><br>*Allyl phenylacetate*<br>CAS n° : 1797-74-6<br><br>*Allyl 3,5,5-trimethylhexanoate*<br>CAS n° : 71500-37-3 | Use only when the level of free allyl alcohol in the ester is less than 0.1%. Based on the delayed irritant potential of allyl alcohol (Food and Chemical Toxicology 15,611(1977)). |
| 4 | *Allyl heptine carbonate*<br><br>CAS n° : 73157-43-4 | Should not be used such that the level in the finished cosmetic products exceeds 0.002 %. Based on test results showing the absence of sensitising reactions for allyl heptine carbonate at low concentrations in a test on humans. This material should not be used in combination with any other 2-alkynoic acid ester (e.g. methyl heptine carbonate). (IFRA guidelines). |
| 5 | *Amylcyclopentenone*<br><br>CAS n° : 25564-22-1 | Should not be used such that the level in the finished cosmetic product exceeds 0.1 %. Based on test results showing sensitisation at 10% and no sensitisation at 1 %. (IFRA guidelines). |

| N° | Substance name | Restriction and condition |
|---|---|---|
| 6 | *Angelica root oil (Angelica archangelica L.)*<br>CAS n° : 8015-64-3<br><br>*Bergamot oil*<br>CAS n° : 8007-75-8<br><br>*Grapefruit oil, expressed (Citrus paradisi Macf.)*<br>CAS n° : 8016-20-4<br><br>*Lemon oil*<br>CAS n° : 8008-56-8<br><br>*Lemon oil, cold pressed, California type*<br>CAS n° : 8008-56-8<br><br>*Lemon oil, cold pressed, desert type*<br>CAS n° : 8008-56-8<br><br>*Lime oil, cold pressed, Mexican*<br>CAS n° : 8008-26-2<br><br>*Lime oil, expressed*<br>CAS n° : 8008-26-2<br><br>*Lime oil expressed rectified*<br>CAS n° : 8008-26-2<br><br>*Orange peel oil, bitter (Citrus aurantium L.)*<br>CAS n° : 68916-04-1<br><br>*Rue oil (Ruta graveolens L.)*<br>CAS n° : 8014-29-7 | May be used in cosmetic products, provided that the total concentration of furocoumarin-like substances in the finished cosmetic product do not exceed 1ppm. |
| 7 | *Balsam oil, Peru (Myroxylon pereirae Klotzsch)*<br>CAS n° : 8007-00-9<br><br>*Balsam absolute, Peru*<br>CAS n° : 8007-00-9<br><br>*Balsam anhydrol, Peru*<br>CAS n° : 8007-00-9 | Extracts and distillates of Peru balsam (the exudation from Myroxylon pereirae (Royle) Klotzsch) should not be used such that the total level exceeds 0.4% in cosmetic products. Based on a wide variety of test results on the sensitising potential of Peru balsam and its derivatives. |
| 8 | *p-tert-Butyldihydro-cinnamaldehyde*<br><br>CAS n° : 18127-01-0 | Should not be used such that the level in the finished cosmetic product exceeds 0.6%. Based on test results showing sensitising potential and no sensitisation reactions when tested at 6%. (IFRA guidelines). |

| N° | Substance name | Restriction and condition |
|---|---|---|
| 9 | Cinnamal<br>CAS n° : 104-55-2<br><br>*Cinnamic aldehyde-methyl anthranilate (Schiff base)*<br>CAS n° : 94386-48-8 | The concentration of Cinnamic aldehyde in the finished cosmetic product should not exceed 0.1%. |
| 10 | *Cassia oil*<br>CAS n° : 8007-80-5<br><br>*Cinnamon bark oil*<br>CAS n° : 8015-91 –6 | The prime allergen is Cinnamic aldehyde. The concentration of Cinnamic aldehyde in the finished cosmetic product should not exceed 0.1%. |
| 11 | Cinnamyl alcohol<br><br>CAS n° : 104-54-1 | Should not be used such that the level in finished cosmetic products exceeds 0.8%. Based on test results showing sensitising potential. (IFRA guidelines). |
| 12 | *Cumin oil*<br><br>CAS n° : 8014-13-9 | For applications on areas of skin exposed to sun, excluding bath preparations, soaps and other wash-off products, limit cumin oil to 0.4% in the finished cosmetic product. Based on the photo-toxicity of cumin oil, the observed no-effect level of 25% on the skin of the hairless mouse and on the no-effect level of 5% in tests with humans. |
| 13 | *cis-.alpha.-Damascone*<br>CAS n° : 23726-94-5<br><br>*trans-. beta.-Damascone*<br>CAS n° : 23726-91-2<br><br>*Isodamascone*<br>CAS n° : 39872-57-6<br><br>*1-(2,6,6-Trimethylcyclohexa-1,3-dienyl)-2-buten-1-one*<br>CAS n° : 23698-85-7<br><br>*1-(2,6,6-Trimethyl-3-cyclohexen-1-yl)-2-buten-l-one*<br>CAS n° : 57378-68-4<br><br>*1-(2,6,6-Trimethyl-l-cyclohexen-1-yl)-2-buten-l-one*<br>CAS n° : 23726-92-3<br><br>*1-(2,6,6-Trimethyl-2-cydohexen-1-yl)-2-buten-l-one*<br>CAS n° : 43052-87-5 | Should not be used as fragrance ingredients such that the total level in finished cosmetic products exceeds 0.02%, individually or in combination. Based on test data showing sensitising potential for these materials and on evidence of cross-reactivity. (IFRA guidelines) |
| 14 | *trans-Hexen-2-al*<br><br>CAS n° : 6728-26-3 | Should not be used such that the level in finished cosmetic products exceeds 0.002%. Based on test results of RIFM showing sensitisation reactions at 0.2% and no sensitisation reactions when tested at 0.02% (IFRA guidelines). |

| N° | Substance name | Restriction and condition |
|---|---|---|
| 15 | Hydroxycitronellal<br><br>CAS n° : 107-75-5 | Should not be used such that the level in finished cosmetic products exceeds 1.0%. Based on a review of a large number of animal and human test data (IFRA guidelines). |
| 16 | Isoeugenol<br><br>CAS n° : 97-54-1 | Should not be used such that the level in finished cosmetic products exceeds 0.02%. Based on test results showing sensitising potential (IFRA guidelines). |
| 17 | *d-Limonene*<br>CAS n° : 5989-27-5<br><br>*l-Limonene*<br>CAS n° : 5989-54-8<br><br>dl-Limonene (racemic)<br>CAS n° : 138-86-3 | Limonene and natural products containing substantial amounts of it, should only be used when the level of peroxides is kept to the lowest practical level, for instance by adding antioxidants at the time of production. Such products should have a peroxide value of less than 20 millimoles peroxide per liter. (IFRA guidelines). |
| 18 | *p-Mentha-1,8-dien-7-al*<br><br>CAS n° : 2111-75-3 | Should not be used such that the level in finished cosmetic products exceeds 0.1%. Based on test results showing sensitisation (IFRA guidelines). |
| 19 | *Menthadiene-7-methyl formate*<br><br>CAS n° : 68683-20-5 | Should not be used such that the level in finished cosmetic products exceeds 0.1 %. Based on tests showing sensitisation (IFRA guidelines). |
| 20 | *Methoxy dicyclopentadiene carboxaldehyde*<br><br>CAS n° : 86803-90-9 | Should not be used such that the level in finished cosmetic products exceeds 0.5%. Based on test results showing sensitisation potential (IFRA guidelines). |
| 21 | *Methyl N-methylanthranilate*<br><br>CAS n° : 85-91-6 | For applications on areas of the skin exposed to sunlight, excluding bath preparations, soaps and other wash-off products, limit to 10**%** in the finished cosmetic product. Based on the phototoxic potential and on the observed no-effect level of approximately 2 mg/cm$^2$ of the hairless mouse (Food and Chemical Toxicology 17, 273 (1979)). |
| 22 | *3-Methyl-2(3)-nonenenitrile*<br><br>CAS n° : 53153-66-5 | Should not be used such that the level in finished cosmetic products exceeds 0.2%. Based on test results showing sensitisation (Food Chemical Toxicology 20, 757 (1982)). |
| 23 | *Methyl octine carbonate*<br>CAS n° : 111-80-8<br><br>*Methyl heptine carbonate*<br>CAS n° : 111-12-6 | Should not be used such that the level in finished cosmetic products exceeds 0.002%.<br><br>When used alone level in the finished cosmetic products should not exceed 0.01%.<br><br>When present in combination, the combined level in the finished product should not exceed 0.01% of which methyl heptine carbonate should not be more than 0.002% |

| N° | Substance name | Restriction and condition |
|---|---|---|
| 24 | *Oakmoss absolute (Evernia spp.)*<br>CAS n° : 9000-50-4<br><br>*Oakmoss resinoid (Evernia spp.)*<br>CAS n° : 9000-50-4<br><br>*Treemoss absolute (Usnea spp.)*<br>CAS n° : 68648-41-9<br><br>*Treemoss concrete (Usnea spp.)*<br>CAS n° : 68648-41-9<br><br>*Treemoss resinold (Usnea spp.)*<br>CAS n° : 868648-41-9 | Should not be used such that the level in finished cosmetic products exceeds 0.1%. The maximum concentration of Oakmoss and Treemoss in combination in the finished cosmetic product should not exceed 0.1%.<br><br>The allergenic fractions in these species have not been fully elucidated nor has the relative contribution of the allergenic fractions between the species. Information needs to be obtained on the nature and the importance of the respective allergenic fractions at which time a review of the acceptance of the restriction in levels and relative proportions of the species will be undertaken. |
| 25 | *1-Octen-3-yl acetate*<br><br>CAS n° : 2442-10-6 | Should not be used such that the level in finished cosmetic products exceeds 0.3%. Based on test results showing sensitising potential (IFRA guidelines). |
| 26 | *3-Propylidenephthalide*<br><br>CAS n° : 17369-59-4 | Should not be used such that the level in finished cosmetic products exceeds 0.01 %. Based on test results showing sensitisation potential (IFRA guidelines). |
| 27 | *2,4,6-Trimethyl-3-cyclohexene-1-methanol*<br><br>CAS n° : 68527-77-5 | Should not be used such that the level in finished cosmetic products exceeds 0.5%. Based on test results showing sensitisation potential (IFRA guidelines). |
| 28 | *Verbena absolute*<br><br>CAS n° : 8024-12-2 | Verbena absolute obtained from Lippia citriodora Kunth should not be used such that the level in finished cosmetic products exceeds 0.2%. Based on test results showing sensitisation (IFRA guidelines). |
| | | |

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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http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm

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## Table of contents

Acknowledgements ..... 3
Table of contents ..... 4
Summary ..... 7
1. Background ..... 9
2. Terms of reference ..... 10
3. Introduction ..... 11
4. Clinical aspects of contact allergy to fragrance ingredients ..... 12
4.1. Spectrum of reactions ..... 12
4.1.1. Allergic contact dermatitis ..... 12
4.1.2. Irritant reactions (including contact urticaria) ..... 14
4.1.3. Pigmentary anomalies ..... 14
4.1.4. Photo-reactions ..... 14
4.1.5. General/respiratory ..... 14
4.2. Patch testing ..... 15
4.3. Epidemiology of fragrance allergy ..... 15
4.3.1. Substances used for screening of contact allergy to fragrance ingredients ..... 15
4.3.2. Clinical epidemiology ..... 16
4.3.3. Population-based epidemiology ..... 23
4.4. Consumer products as a cause of fragrance contact sensitisation and allergic contact dermatitis ..... 25
4.4.1. Clinical relevance ..... 25
4.4.2. Elicitation with clinical symptoms/signs, current and past ..... 26
4.4.3. Elicitation in diagnostic patch tests without clinical history ..... 28
4.5. Socio-economic impact of contact allergy ..... 29
4.5.1. Health related quality of life ..... 29
4.5.2. Occupational restrictions ..... 29
4.5.3. Costs to health care/health economics ..... 29
4.6. Allergen avoidance ..... 30
4.6.1. Primary prevention: limiting or eliminating exposure to allergens in the population ..... 30
4.6.2. Secondary prevention: avoiding re-exposure to (a) specific sensitiser(s) in clinically diagnosed individuals ..... 30
4.7. Conclusions ..... 32
5. Activation of weak or non-sensitising substances into sensitisers - prehaptens and prohaptens ..... 33
5.1. Prehaptens ..... 33
5.2. Prohaptens ..... 37
5.3. Conclusions ..... 39
6. Retrieval of evidence and classification of fragrance substances ..... 40
6.1. Retrieval of evidence ..... 40

6.1.1. Search strategy for clinical data ..... 40
6.1.2. Collection of experimental (LLNA) data ..... 41
6.2. Grading of evidence ..... 41
6.2.1. Quality of a clinical study ..... 41
6.2.2. Quality of an experimental study ..... 42
6.2.3. Quality of "other" evidence ..... 42
6.3. Aggregating evidence for a final conclusion ..... 42
6.3.1. Established contact allergen in humans ..... 42
6.3.2. Established contact allergen in animals ..... 43
6.3.3. Likely contact allergen, if human, animal and other evidence is considered ... 43
6.3.4. Possible contact allergen, if human, animal and other evidence is considered 43
6.4. Conclusions ..... 44
7. Reported fragrance allergens from the clinical perspective ..... 45
7.1. Tabular summary of evaluated individual fragrance chemicals ..... 45
7.2. Tabular summary of evaluated natural extracts/essential oils ..... 53
7.3. Conclusions ..... 57
8. Animal data ..... 58
8.1. Predictive tests and sensitising potency categories ..... 58
8.1.1. LLNA data ..... 59
8.1.2. LLNA data on oxidised fragrance substances ..... 61
8.2. Methodological considerations ..... 62
8.3. Summary of animal data by LLNA ..... 63
8.4. Conclusions ..... 64
9. Structure activity relationships (SAR): grouping of substances based on expert judgement ..... 66
9.1. General results ..... 71
9.2. Conclusions ..... 71
10. Exposure ..... 72
10.1. Concentrations and quantities used ..... 72
10.2. Global exposure (household and occupational exposures) ..... 81
10.3. Exposures related to particular anatomical sites ..... 84
10.4. Conclusion ..... 86
11. Dose-response relationships and thresholds ..... 87
11.1. Induction ..... 87
11.2. Elicitation ..... 88
11.2.1. General considerations ..... 88
11.2.2. Studies on specific fragrance ingredients ..... 90
11.3. Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) ..... 98
11.4. Conclusion ..... 101
12. Data gaps and research needed ..... 103
12.1. Clinical and epidemiological research ..... 103

12.2. Non-human studies ..... 104
13. Opinion ..... 105
13.1. Question 1 ..... 106
Conclusions - Question 1 ..... 114
13.2. Question 2 ..... 115
Conclusions - Question 2 ..... 116
13.3. Question 3 ..... 117
Conclusions - Question 3 ..... 119
14. List of abbreviations ..... 121
15. References ..... 123
Annex I - Catalgoue of fragrance allergens ..... 141
Single chemicals ..... 142
Catalogue of single chemicals evaluated ..... 146
Natural extracts / essential oils ..... 237
Catalogue of natural extracts / essential oils evaluated ..... 238
References ..... 277
Annex II - Animal Data ..... 293
References ..... 309
Annex III - Tabular summary of dose-elicitation studies in sensitised patients ..... 315
Chloroatranol ..... 316
Cinnamal ..... 318
Hydroxycitronellal ..... 321
Hydroxyisohexyl 3-cyclohexenecarboxaldehyde (HICC) ..... 323
Isoeugenol ..... 329
References ..... 333

## 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<br>2006 | *Myroxylon pereirae* | 567<br>3460 | 1.1<br>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 10$^{th}$ 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.);<br>+++ (ox.) |
| LINALOOL | 78-70-6 | ++ (non-ox.)<br>+++ (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"<br>0 / 95 in another study with <= 1/10 of above test conc. | (139)<br>(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<br>0 / 100 | (97)<br>(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;<br>0 / 100 | (139)<br>(70) |
| PHENYLACETALDEHYDE | 122-78-1 | 1.1% of 182 positive.<br>1 case | (178)<br>(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<br>"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<br>"top 100" substance | (70) |
| ISOBORNYL ACETATE | 125-12-2 | 0 / 107<br>"top 100" substance | (70) |
| METHYL ANTHRANILATE | 134-20-3 | 0 / 91<br>"top 100" substance | (188) |
| METHYL IONONE (mixture of isomers) | 1335-46-2 | 0 / 100<br>"top 100" substance | (70) |
| OXALIDE | 1725-01-5 | 0 / 178 | (171) |
| TERPINEOL ACETATE (Isomer mixture) | 8007-35-0 | 0 / 106<br>"top 100" substance | (70) |
| alpha-TERPINYL ACETATE | 80-26-2 | 0 / 179 | (139) |
| TRIMETHYL-PROPYLCYCLOHEXANEPROPANOL | 70788-30-6 | 0 / 178 | (171) |

For yet another subset of substances, no human data were publicly available. However, exposure to these substances is important as they are used in high volumes (this being the sole criterion for inclusion in this list) and, therefore their hazard with regard to contact sensitisation should be examined.

**Table 7-4:** Fragrance substances lacking human data and used in high volumes according to industry information.

| **INCI name (or, if none exists, perfuming name according to CosIng)** | **CAS number** |
|---|---|
| ANISALDEHYDE | 123-11-5 |
| BENZYL ACETONE | 2550-26-7 |
| p-tert. -Butyldihydrocinnamaldehyde | 18127-01-0 |
| CITRONELLYL NITRILE | 51566-62-2 |
| CYCLAMEN ALDEHYDE | 103-95-7 |
| alpha-CYCLOHEXYLIDENE BENZENEACETONITRILE | 10461-98-0 |
| DECANAL | 112-31-2 |
| DIHYDROMYRCENOL | 18479-58-8 |
| 2,4-DIMETHYL-3-CYCLOHEXEN-1-CARBOXALDEHYDE | 68039-49-6 |
| 3,7-DIMETHYL-1,6-NONADIEN-3-OL | 10339-55-6 |
| DIPHENYL ETHER | 101-84-8 |
| ETHYL 2-METHYLBUTYRATE | 7452-79-1 |
| 2-ETHYL-4-(2,2,3-TRIMETHYL-3-CYCLOPENTEN-1-YL)-2-BUTEN-1-OL | 28219-61-6 |
| ETHYLENE BRASSYLATE | 105-95-3 |
| EUCALYPTOL | 470-82-6 |
| GERANYL ACETATE | 105-87-3 |
| HEXAHYDRO-METHANOINDENYL PROPIONATE | 68912-13-0 |
| HEXYL ACETATE | 142-92-7 |
| IONONE isomeric mixture | 8013-90-9 |
| ISOAMYL ACETATE | 123-92-2 |
| ISOBERGAMATE [#] | 68683-20-5 |
| Longifolene | 475-20-7 |
| METHYLENEDIOXYPHENYL METHYLPROPANAL | 1205-17-0 |
| METHYLBENZYL ACETATE | 93-92-5 |
| METHYL DECENOL | 81782-77-6 |
| METHYL beta-NAPHTHYL ETHER | 93-04-9 |
| METHYLUNDECANAL | 110-41-8 |
| OXACYCLOHEXADECENONE | 34902-57-3 |
| PENTADECALACTONE | 106-02-5 |
| PHENETHYL ACETATE | 103-45-7 |
| PHENOXYETHYL ISOBUTYRATE | 103-60-6 |
| PHENYLISOHEXANOL | 55066-48-3 |
| Tetrahydrolinalool | 78-69-3 |
| TETRAHYDRO-METHYL-METHYLPROPYL)-PYRAN-4-OL | 63500-71-0 |

[#] Annex III, part 1

| INCI name (or, if none exists, perfuming name according to CosIng) | CAS number |
|---|---|
| TRICHLOROMETHYL PHENYL CARBINYL ACETATE | 90-17-5 |
| TRICYCLODECENYL PROPIONATE | 17511-60-3 |
| TRIMETHYLHEXYL ACETATE | 58430-94-7 |
| gamma-UNDECALACTONE | 104-67-6 |
| VERDYL ACETATE | 2500-83-6/ 5413-60-5 |

### 7.2. Tabular summary of evaluated natural extracts/essential oils

Natural raw materials in terms of extracts are used in the fragrance and flavour industry for various reasons. Most importantly, several naturally occurring mixtures have a very complex composition and sensory nature which cannot (fully) be achieved by synthetic the demand for perfumes based on natural materials is considerable (189).

The three main methods used to concentrate plant fragrance substances (190); distillation, mechanical separation ("pressing"), and solvent extraction, yield very different extracts. Essential oils are obtained by water steam, water, ethanol, or water/ethanol distillation. Essence oils are essential oils that separate from the aqueous phase in the distillation receiver during the distillative concentration of fruit, usually citrus, juices. Citrus peel oils, apart from distilled lime oil, are prepared in a special way by pressing the peel to release mostly volatile substances from the pericarp in small oil glands, mostly highly volatile terpene hydrocarbons. However, they also contain small amounts of non-volatile compounds such as dyes, waxes and furocoumarins. The method of solvent extraction is generally applied in the separation of heat-labile materials or if an essential oil can only be obtained in very low yield, e.g. from blossoms. It is also used if the non-volatile components are desired for their fixative properties, e.g. in the preparation of resinoids from exudates. The most important extracts are termed: (i) concretes, an extract of fresh plant material with nonpolar solvents, containing not only volatile, but also a large proportion of non-volatile substances such as waxes; and (ii) absolutes, which are prepared by taking up concretes in ethanol; compounds that precipitate on cooling are removed by filtration, yielding a wax-free residue called absolute. Resinoids, used for their fixative properties, are prepared by extracting plant exudates with alcohols or nonpolar solvents. The products are usually highly viscous and thus sometimes diluted, e.g. with phthalates or benzyl benzoate. Oleoresins are concentrates prepared from spices by solvent extraction (189).

An ISO norm exists regarding the nomenclature of aromatic natural raw materials (ISO/DIS 9235 Aromatic raw materials - vocabulary; International Standardisation Organisation, Geneva, Switzerland). This nomenclature has been considered in Annex I, whereas in the present opinion, nomenclature is according to the CosIng database. Concerning extraction processes for many essential oils, ISO standards exist; for detailed information see Annex I to this opinion.

Regarding clinical data in terms of contact allergy to essential oils and natural extracts, the main focus is on general dermatological patients with complaints related to use of cosmetics etc. However, series of cases with occupational exposure to essential oils with occupational allergic contact dermatitis have also been reported (e.g. masseurs, physiotherapists (191, 192), aromatherapists (193-197), beauticians performing massages (198). For further details, e.g. PT results with various essential oils, see Annex I.

In this section, a tabular overview on the classification of substances considered is presented in three tables listing:

1. Extracts identified as *established contact allergens* in humans(→ *sufficient human evidence present*).

2. Extracts with positive human data, which are, however, not sufficient to categorise as *established contact allergen* in humans (→ *limited human evidence present*).
3. Extracts with negative human data, i.e. patch tests of patients with suspected contact allergy to fragrance ingredients which yielded negative results.

In Table 7-5, essential oils with sufficient human evidence to categorise these as *established contact allergens* in humans are presented.

**Table 7-5:** Natural extracts classified as established contact allergens in humans (summary of evaluation as detailed in chapter 6.3). More detailed information forming the basis of this evaluation can be found in Annex I of this opinion, including variants of botanical nomenclature.

| **INCI name (or, if none exists, $perfuming name according to CosIng[4]) in bold;** plant part / type of extract (partly indicative) in plain font | **CAS number** | **Comment: see text** |
|---|---|---|
| ***CANANGA ODORATA and Ylang-ylang oil*** | 83863-30-3; *8006-81-3* | +++ |
| ***CEDRUS ATLANTICA*** *BARK OIL* | *92201-55-3; 8000-27-9* | ++ |
| ***CINNAMOMUM CASSIA*** *LEAF OIL* ***CINNAMOMUM ZEYLANICUM*** *BARK OIL* | *8007-80-5* *84649-98-9* | ++ (r.t.) |
| ***CITRUS AURANTIUM AMARA*** *FLOWER / PEEL OIL* | *8016-38-4; 72968-50-4* | ++ |
| ***CITRUS BERGAMIA*** *PEEL OIL EXPRESSED*$ | *89957-91-5* | + (r.t.) |
| ***CITRUS LIMONUM*** *PEEL OIL EXPRESSED* # | *84929-31-7* | ++ |
| ***CITRUS SINENSIS*** *(syn.: AURANTIUM DULCIS) PEEL OIL EXPRESSED*$ | *97766-30-8; 8028-48-6* | ++ |
| ***CYMBOPOGON CITRATUS*** / ***SCHOENANTHUS*** *OILS* | *89998-14-1; 8007-02-1; 89998-16-3* | ++ |
| ***EUCALYPTUS SPP.*** *LEAF OIL*$ | *92502-70-0; 8000-48-4* | ++ |
| ***EUGENIA CARYOPHYLLUS*** *LEAF / FLOWER OIL* | *8000-34-8* | +++ |
| ***EVERNIA FURFURACEA*** *EXTRACT [5](Tree moss)* | *90028-67-4* | +++ |
| ***EVERNIA PRUNASTRI*** *EXTRACT (Oak moss)* # | *90028-68-5* | +++ |
| ***JASMINUM GRANDIFLORUM / OFFICINALE*** | *84776-64-7; 90045-94-6; 8022-96-6* | +++ |
| ***JUNIPERUS VIRGINIANA*** | *8000-27-9; 85085-41-2* | ++ |
| ***LAURUS NOBILIS*** | *8002-41-3; 8007-48-5; 84603-73-6* | ++ |
| ***LAVANDULA HYBRIDA*** | *91722-69-9* | + (r.t.) |
| ***LAVANDULA OFFICINALIS***$ | *84776-65-8* | ++ |
| ***MENTHA PIPERITA*** | *8006-90-4; 84082-70-2* | ++ |
| ***MENTHA SPICATA*** | 84696-51-5 | ++ |
| ***MYROXYLON PEREIRAE****(Balsam of Peru)* # | 8007-00-9 | ++++ |

[4] http://ec.europa.eu/consumers/cosmetics/cosing/

# 76/768/EEC Annex III, part 1

# 76/768/EEC Annex III, part 1

| **INCI name (or, if none exists, $perfuming name according to CosIng[4]) in bold;** plant part / type of extract (partly indicative) in plain font | **CAS number** | **Comment: see text** |
|---|---|---|
| ***NARCISSUS SPP.*** | *diverse* | ++ |
| ***PELARGONIUM GRAVEOLENS*** | *90082-51-2; 8000-46-2* | ++ |
| ***PINUS MUGO/ PUMILA*** # | *90082-72-7 / 97676-05-6* | ++ |
| ***POGOSTEMON CABLIN*** | *8014-09-3; 84238-39-1* | ++ |
| ***ROSE FLOWER OIL (ROSA SPP.)*** | *Diverse* | ++ |
| ***SANTALUM ALBUM*** | *84787-70-2; 8006-87-9* | +++ |
| ***TURPENTINE*** *(oil)* # | 8006-64-2; 9005-90-7; 8052-14-0 | ++++ |
| **VERBENA absolute** # | 8024-12-2 | ++ |

Notes: r.t. Rarely tested.

Table 7-6 lists a number of essential oils, mostly tested in just one clinical department, and thus, or for other reasons, not satisfying the criteria for being categorised as *established contact allergen* in humans (i.e. *limited human evidence present*).

**Table 7-6:** Natural extracts with positive human data, which are, however, not sufficient to categorise as "established contact allergen in humans". More detailed information forming the basis of this evaluation can be found in Annex I of this opinion.

| **INCI name (or, if none exists, perfuming name according to CosIng) in bold;** plant part / type of extract (partly indicative) in plain font | **CAS number** | **Comment** | **Ref.** |
|---|---|---|---|
| ***ACORUS CALAMUS*** *ROOT OIL* | *84775-39-3* | n=7 pos. reactions to "calamus" | (199) |
| ***CEDRUS DEODARA*** *WOOD OIL* | *91771-47-0* | Rudzki 1976/1986 found 3 / 3 positive reactions | (199, 200). |
| ***CITRUS AURANTIUM AMARA*** *LEAF OIL* | *72968-50-4* | Several cases in 2 series from 1 centre | (199, 200) |
| ***CITRUS TANGERINA*** *…* | *223748-44-5* | 1 case | (201) |
| ***CYMBOPOGON NARDUS / WINTERIANUS*** *HERB OIL* | *89998-15-2; 91771-61-8* | Several cases in 2 series from 1 centre | (199, 200) |
| ***ILLICIUM VERUM*** *FRUIT OIL* | *84650-59-9* | Cases of active sensitisation; 34% consecutive patients pos. to 1% | (202) |

| **INCI name (or, if none exists, perfuming name according to CosIng) in bold;** plant part / type of extract (partly indicative) in plain font | **CAS number** | **Comment** | **Ref.** |
|---|---|---|---|
| ***LAVANDULA SPICA*** | *97722-12-8* | Several cases in 2 series from 1 centre | (199, 200) |
| ***LITSEA CUBEBA*** | *90063-59-5* | Several cases in 2 series from 1 centre | (199, 200) |
| ***PELARGONIUM ROSEUM*** | *90082-55-6* | 2.1% pos. of 1483 patients | (203) |
| ***ROSMARINUS OFFICINALIS*** | *84604-14-8* | 3 cases in 2 series from 1 centre | (199, 200) |
| ***SALVIA spp.*** | *Diverse* | Several cases in 2 series from 1 centre | (199, 200) |
| ***TAGETES PATULA*** | *91722-29-1* | 1 case (aromatherapist) | (193) |
| ***THYMUS spp.*** | *84929-51-1* | 4 / 84 pos | (199) |
| ***VETIVERIA ZIZANOIDES*** | *8016-96-4; 84238-29-9* | 1 / 200 and 9 / 86 pos. | (199, 200) |

The final table is an indicative list of natural extracts which lack published human data, but which are of interest: (i) as high-volume exposure; (ii) due to published positive animal experiments; or (iii) because they contain well-known (established) contact allergens.

**Table 7-7:** Indicative list illustrating natural extracts containing established human allergens or having R43-lable or positive LLNA, lacking published human data.

| **INCI name (or, if none exists, perfuming name according to CosIng) in bold;** plant part / type of extract (partly indicative) in plain font | **CAS number** | **Comment** |
|---|---|---|
| ***CITRUS PARADISI*** *PEEL OIL* | 8016-20-4 | high volume substance, classified as R43 |
| ***CYMBOPOGON MARTINI*** *HERB EXTRACT* | *84649-81-0* | Pos. LLNA study by RIFM: EC3 value 9.6% (204). |
| ***MENTHA ARVENSIS*** | 68917-18-0 | high volume, classified as R43 |
| ***OCIMUM BASILICUM*** | *84775-71-3* | Pos. LLNA study by RIFM: EC3 value < 2.5% (204). |
| *PIMENTA RACEMOSA* | 85085-61-6 | Contains, among other substances, the established contact allergen eugenol (42-56%) |
| ***SANTALUM SPICATA*** | *8024-35-9* | Contains, among other substances, the established contact allergens santalols (75%) and farnesol (10%) |

### 7.3. Conclusions

- According to the criteria described in chapter 6.3 a total of 54 individual chemicals and 28 natural extracts (essential oils) can be categorised as *established contact allergens* in humans, including all currently regulated substances.
- Of the 54 individual chemicals which are established contact allergens in humans, 12 are considered to be of special concern due to the high number of reported cases, (> 100, i.e. category +++ or ++++ in Table 7-1). These are further considered in chapter 5 (limonene and linalool) and the remainder in chapter 11. In particular one ingredient stands out, hydroxyisohexyl 3-cyclohexene carboxaldehyde, having been the cause of more than 1,500 reported cases since the 1999 opinion (see also chapter 4.3.1, chapter 11.3 and Annex I).
- For an additional 33 individual chemicals (Table 7-2) and 14 natural extracts (Table 7-6), positive patch test results have been reported. However, they do not qualify for the above category, i.e.only *limited human evidence* is present.
- For a number of fragrance substances (n=18, Table 7-3) patch testing did not yield positive results. However, numbers of patients tested are generally too small to rule out the existence of clinical contact sensitisation with sufficient confidence.No clinical evidence has been identified for 39 individual chemicals that have been reported to be frequently used (Table 7-4).
- For the substances (and, if possible, also for the main constituents of the natural mixtures) with limited or no human evidence, additional animal data and/or SAR considerations are taken into account. Aggregated data for these substances are presented in chapter 13.

# 8. Animal data

## 8.1. Predictive tests and sensitising potency categories

The animal test methods used in harmonised classification of substances, according to their potential to cause skin sensitisation, are the guinea pig maximisation test (GPMT), the Buehler test[6] and the local lymph node assay (LLNA)[7]. These methods are used in hazard identification and risk assessment for regulatory purposes under REACH[8]. For registration in REACH, the LLNA is the preferred method for measuring skin sensitisation potential in animals, and justification for the use of other methods needs to be provided. According to the directives on classification and labelling[9], substances and preparations meeting positive criteria in these tests shall be classified as sensitising and assigned the symbol "Xi" and the risk phrase "R43: May cause sensitisation by skin contact"; or, according to the recent regulation on classification, labelling and packaging (CLP[10]) "H317: May cause an allergic skin reaction".

As yet, there is no officially validated *in vitro* test method for skin sensitisation. Therefore, for cosmetic ingredients the LLNA, the GPMT and the Buehler test have also been used in risk assessment for regulatory purposes.

Positive results from the OECD guideline animal tests mentioned above which are sufficient to classify a substance as a skin sensitiser (R43) are:

- GPMT; at least 30% of the animals have a positive response.
- Buehler test; at least 15% of the animals have a positive response.
- LLNA; at least a 3-fold increase in lymph node cell proliferative activity is induced, compared to vehicle-treated controls (stimulation index SI ≥3). For positive LLNAs, an EC3 value is calculated which gives the estimated concentration of a chemical necessary to give a 3-fold increase in proliferative activity compared to vehicle-treated controls.

Further categorisation of substances classified with R43 into three groups according to allergen potency (extreme, strong and moderate) has been proposed by a European Commission expert group on skin sensitisation (161, 205), and proposed also in the ECHA guidance document on application of the CLP criteria (162). Such categorisation is based on EC3 values in the LLNA, on intradermal induction concentration in the GPMT, and topical induction concentration in the Buehler test. The potency categories and their default concentration values based on EC3 values in the LLNA as defined in (161): extreme sensitiser (EC3 value ≤ 0.2); strong sensitiser (EC3 > 0.2 - ≤ 2); and moderate sensitiser (EC3 value > 2). When LLNA EC3 values are available from more than one study, the lowest value should normally be used. Where multiple animal data sets lead to different categorisation of the same substance, the higher potency category should apply (161, 205).

The potency categorisation of substances based on the LLNA is applied by the SCCP in risk assessment of cosmetic ingredients, particularly hair dye substances (206).

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[6] OECD Guideline for testing of chemicals. Guideline 406: Skin Sensitisation. OECD, Adopted 12 May 1981, updated 17th July 1992.

[7] OECD Guideline for testing of chemicals. Guideline 429: Skin Sensitisation: Local Lymph Node Assay. OECD, Adopted 22 July 2010.

[8] Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).

[9] Directives 67/548/EEC and 1999/45/EC.

[10] Regulation No. 1272/2008.

### 8.1.1. LLNA data

The SCCS requested the International Fragrance Association (IFRA) to submit data on animal tests performed with fragrance substances, to be presented in a structured format. In response, IFRA submitted first a poster (163) and later a report consisting of LLNA protocol summaries on the 59 fragrance substances in the poster (164). No guinea pig studies were submitted. The SCCS has reviewed and analysed the report and the publications quoted in the report.

Table 8-1 displays the EC3 values for fragrance substances in the report submitted by industry (164). EC3 values for some additional fragrance substances in two published reviews (165, 166) have also been included in Table 8-1. Table 8-2 presents LLNA results for oxidised substances. Full data are given in Annex II. Table 8-3 summarises the distribution of fragrance substances, by potency category, according to EC3 values.

Additional EC3 values may be available in the scientific literature. Many more animal experiments may have been performed, but have not been published.

**Table 8-1:** Summary of local lymph node assay (LLNA) data on 66 fragrance substances, based on a report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009 (164)) and in published reviews by Gerberick et al. 2005 (165) and Kern et al. 2010 (166), respectively. EC3 values (% and M) are given. The order of substances is by decreasing sensitisation potency as assessed by LLNA EC3 values (lowest EC3 value indicating highest potency).

| **Substance** | **CAS no.** | **EC3 value** | | **Reference** |
|---|---|---|---|---|
| | | **%** | **M** | |
| Hexyl salicylate | 6259-76-3 | 0.18 | 0.008 | (164, 166) |
| Cinnamal | 104-55-2 | 0.2 | 0.015 | (164) |
| Methyl 2-octynoate | 111-12-6 | <0.5 | <0.032 | (164, 166) |
| Isoeugenol | 97-54-1 | 0.54 | 0.033 | (164) |
| Citral | 5392-40-5 | 1.2 | 0.079 | (164) |
| 2-Hexylidene cyclopentanone | 17373-89-6 | 2.4 | 0.14 | (164) |
| Methyl octine carbonate | 111-80-8 | 2.5 | 0.15 | (164) |
| Peru balsam absolute | 8007-00-9 | 2.5 | n/a | (164) |
| trans-2-Hexenal | 6728-26-3 | 2.6 | 0.26 | (164) |
| Benzyl Salicylate | 118-58-1 | 2.9 | 0.23 | (164, 166) |
| Butylphenyl methylpropional (BMHCA) | 80-54-6 | 2.9 | 0.14 | (164) |
| Phenylacetaldehyde | 122-78-1 | 3 | 0.25 | (164, 165) |
| Allyl phenoxyacetate | 7493-74-5 | 3.1 | 0.16 | (164) |
| Benzylideneacetone | 122-57-6 | 3.7 | 0.25 | (165) |
| 3-Propylidenephthalide | 17369-59-4 | 3.7 | 0.21 | (164, 165) |
| *Evernia prunastri* extract oak moss | 90028-68-5 | 3.9 | n/a | (164) |
| Balsam oil, Peru *(Myroxylon pereirae Klotzsch)* | 8007-00-9 | 4 | n/a | (164) |
| Farnesol | 4602-84-0 | 4.1 | 0.18 | (164) |
| p-t-Butyl-dihydrocinnamaldehyde | 18127-01-0 | 4.3 | 0.23 | (164) |
| α-Methyl cinnamic aldehyde | 101-39-3 | 4.5 | 0.31 | (164, 165) |

| Substance | CAS no. | EC3 value | | Reference |
|---|---|---|---|---|
| | | % | M | |
| Eugenol | 97-53-0 | 5.3 | 0.32 | (164) |
| Hexyl cinnamal | 101-86-0 | 5.3 | 0.25 | (164) |
| Dihydrocoumarin | 119-84-6 | 5.6 | 0.38 | (165) |
| Geraniol | 106-24-1 | 5.6 | 0.36 | (164) |
| Carvone | 6485-40-1 | 5.7 | 0.38 | (164) |
| Diethyl maleate | 141-05-9 | 5.8 | 0.34 | (165) |
| 2-Methoxy-4-methylphenol | 93-51-6 | 5.8 | 0.42 | (164, 165) |
| Anise alcohol | 105-13-5 | 5.9 | 0.43 | (164, 166) |
| Jasmine absolute *(Grandiflorum)* | 8022-96-6 | 5.9 | N/a | (164) |
| Dibenzyl ether | 103-50-4 | 6.3 | 0.32 | (164) |
| *Cananga odorata* leaf/flower oil ylang ylang "extra" | 8006-81-3 | 6.8 | N/a | (164) |
| Isocyclocitral | 1335-66-6 | 7.3 | 0.48 | (164) |
| 2,3-Dihydro-2,2,6-trimethylbenzaldehyde | 116-26-7 | 7.5 | 0.50 | (165) |
| Amyl cinnamal | 122-40-7 | 7.6 | 0.38 | (164) |
| Perillaldehyde p-Mentha-1,8-dien-7-al | 2111-75-3 | 8.1 | 0.54 | (164, 165) |
| p-Isobutyl-α-methyl hydrocinnamaldehyde | 6658-48-6 | 9.5 | 0.46 | (164) |
| d-Limonene* | 5989-27-5 | <10 | <0.73 | (164) |
| Methylundecanal | 110-41-8 | 10 | 0.54 | (165) |
| Acetylcedrene | 32388-55-9 | 13.9 | 0.57 | (166) |
| Methylenedioxyphenyl methylpropanal | 1205-17-0 | 16.4 | 0.85 | (164, 166) |
| Benzyl benzoate | 120-51-4 | 17 | 0.80 | (165) |
| Hydroxyisohexyl 3-cyclohexene carboxaldehyde | 31906-04-4 | 17.1 | 0.81 | (164, 165) |
| Benzyl cinnamate | 103-41-3 | 18.4 | 0.77 | (164, 166) |
| Hydroxycitronellal | 107-75-5 | 19.3 | 1.12 | (164) |
| Cinnamyl alcohol | 104-54-1 | 21 | 1.57 | (165) |
| α-iso-Methylionone | 127-51-5 | 21.8 | 1.06 | (164, 166) |
| Cyklamen aldehyde | 103-95-7 | 22 | 1.64 | (165) |
| 4-Methoxy-α-methyl benzenpropanal | 5462-06-6 | 23.6 | 1.32 | (164) |
| Amyl cinnamyl alcohol | 101-85-9 | ~25 | ~1.22 | (164, 166) |
| Tetramethyl acetyloctahydronaphthalenes (OTNE) | 54464-57-2 | 25.1 | 1.07 | (164) |
| Ethyl acrylate | 140-88-5 | 28 | 2.8 | (165) |
| Linalool* | 78-70-6 | 30 | 1.94 | (165) |
| Trimethylbenzenepropanol Majantol | 103694-68-4 | 30 | ~1.68 | (164) |
| Jasminum Sambac Flower CERA/Extract/Water | 91770-14-8 | 35.4 | N/a | (164) |

| Substance | CAS no. | EC3 value | | Reference |
|---|---|---|---|---|
| | | % | M | |
| Citronellol | 106-22-9 | 43.5 | 2.78 | (164, 166) |
| **No EC3 value was established; higher concentrations should also have been tested** | | | | |
| 6-Methyl-3,5-heptadien-2-one | 1604-28-0 | >5 | >0.40 | (164) |
| *Camellia sinensis* leaf tea leaf absolute | 84650-60-2 | >5 | N/a | (164) |
| Cinnamyl nitrile | 1885-38-7 | >10 | >0.77 | (164) |
| Menthadiene-7-methyl formate | 68683-20-5 | >10 | >0.51 | (164) |
| *Evernia furfuracea* extract tree moss absolute | 90028-67-4 | >20 | N/a | (164) |
| Isocyclogeraniol | 68527-77-5 | >25 | >1.62 | (164) |
| 1-Octen-3-yl acetate | 2442-10-6 | >30 | >1.76 | (164) |
| Benzyl alcohol | 100-51-6 | >50 | >4.62 | (164) |
| Coumarin | 91-64-5 | >50 | >3.42 | (164) |
| Vanillin | 121-33-5 | >50 | >3.3 | (164) |
| **No EC3 value calculated** | | | | |
| Benzaldehyde | 100-52-7 | - | | (165) |

Notes: * Material with low levels of oxidation according to (164)
n/a: Not applicable (mixture of compounds).

M: EC3 based on molar concentration

### 8.1.2. LLNA data on oxidised fragrance substances

For fragrance substances that can autoxidise upon air exposure, it is also important to investigate the sensitisation potency after air exposure. The oxidised compounds are clinically relevant as they represent what the consumers could come in contact with from perfumes and fragranced products. In Table 8-2 the LLNA data for some of the most commonly used fragrance substances, pure and after autoxidation, are presented. The EC3 values obtained for the pure substances are 5-10 times higher compared to those obtained for the same substances after air exposure. The experimental air exposure simulated air exposure that can take place during normal handling and storage. In the production process, some perfumes are "matured" aerobically, stirring included. During this process, some fragrance substances may be oxidised. It should be noted that, although only a few substances capable of oxidation have so far been investigated, structural alerts indicating possible autoxidation are common among the fragrance substances listed in this document (see chapter 9). It is important to further investigate this issue for increased understanding of the associated risk.

**Table 8-2:** Local lymph node assay (LLNA) data on four fragrance substances and one essential oil before and after air exposure, comparing the sensitisation potency of the pure (not oxidised) substance with the potency of the oxidised.

| Substance | CAS no. | Doses % (w/v) vehicle: A:OO 4:1* | EC3 value (% w/v) | Reference |
|---|---|---|---|---|
| D-Limonene (ox. 10 w) | 5989-27-5 | 1, 5, 25 | 3.0 | (207) |
| D-Limonene (pure) | 5989-27-5 | 25, 50, 100 | 30 | |

| Substance | CAS no. | Doses % (w/v) vehicle: A:OO 4:1* | EC3 value (% w/v) | Reference |
|---|---|---|---|---|
| Linalool (ox. 10 w) | 78-70-6 | 5, 10, 25 | 9.4 | (127) |
| Linalool (ox. 45 w) | 78-70-6 | 2.5, 10, 25 | 4.8 | |
| Linalool (pure) | 78-70-6 | 25, 50, 100 | 46.2 | |
| Linalyl acetate (ox. 10 w) | 115-95-7 | 0.5, 10, 40 | 3.6 | (128) |
| Linalyl acetate (pure) | 115-95-7 | 10, 30, 100 | 25 | |
| Geraniol (ox. 10 w) | 106-24-1 | 1, 3, 6, 10, 20 | 4.4 | (119) |
| Geraniol (ox. 45 w) | 106-24-1 | 0.5, 1, 3, 6, 10 | 5.8 | |
| Geraniol (pure) | 106-24-1 | 5, 10, 15, 20, 30 | 22.4 | |
| Lavender oil (ox. 10 w) | | 1, 5, 10, 20, 50 | 11 | (140) |
| Lavender oil (ox. 45 w) | | 1, 5, 10, 20, 50 | 4.4 | |
| Lavender oil (not ox.) | | 5, 25, 100 | 36 | |

Notes: Pure: Purified before testing as most commercially available fragrance substances are not pure.
Not ox.: Not purified but used as it was delivered as this is a complex mixture and not a specific substance.
Ox. x w: Oxidised by air exposure during x weeks.
* Acetone:olive oil.

### 8.2. Methodological considerations

***EC3 mean values***

In the submitted poster (163) and the report by IFRA (164), the LLNA weighted mean EC3 values (µg/cm$^2$) are presented. The SCCS considers it is misleading to present EC3 values as mean values from tests performed with different vehicles. It is generally agreed that the lowest EC3 value should be used if there is more than one study fulfilling the OECD guideline requirements (161, 205), and these have been introduced into Table 8-1. The EC3 values in the reviews by Gerberick et al. and Kern et al. (165, 166) were based on single representative experiments with a vehicle described in the OECD guideline 429 (see above), and preferably with acetone:olive oil. EC3 mean values, as in the submission by IFRA, were not presented in these two reviews.

***Vehicle***

The most frequently used *vehicle* in the submission by IFRA (164) was ethanol:diethyl phthalate (1:3), followed by acetone:olive oil (4:1). In some experiments, antioxidants were mixed with ethanol:diethyl phthalate. The vehicle was not reported in some of the references, and no rationale for using vehicles other than those recommended was given in the report (164). According to the OECD guideline 429 (see above), the recommended vehicles are acetone:olive oil (4:1), N,N-dimethylformamide, methyl ethyl ketone, propylene glycol, and dimethyl sulphoxide, but others may be used if sufficient scientific rationale is provided. It is well known that a difference in the EC3 value can be obtained for the same substance depending on which vehicle is used in the LLNA. Thus as an *additional control*, supplementary to the guideline based LLNA control, a clinically relevant solvent or the commercial formulation in which the test substance is marketed may be used.

***Number of doses and animals***

According to the OECD guideline 429 (see above), a minimum of three concentrations should be tested. The number of consecutive doses used in the reported data, was generally five, sometimes three and in few experiments two. The SCCS considers that too few concentrations were tested in four studies in which only two concentrations were used. Lower concentrations than those tested should have been used in experiments with five fragrance substances, in which the EC3 value could not be determined. Higher concentrations than those tested should also have been used in experiments with 12 substances, in which the EC3 value could not be determined.

The *number of animals* per dose group was generally four plus a non-exposed control group, sometimes five, and in few experiments six; the minimum according to the OECD guideline being four.

***Units for concentrations***

In the submission by IFRA (164) the EC3 values are given in weight per area unit (μg/cm$^2$). The SCCS considers that the EC3 values (%) are the values of primary interest in communicating risk assessment, as EU legislation, OECD guideline 429 and scientific literature refer to EC3 values (%). However, the SCCS recommends that molar (M) EC3 values should be considered, as they give the concentration based on the molecular weight of substances. They have thus been calculated and introduced into Table 8-1.

EC3 values (%) overestimate the intrinsic molecular sensitisation potency for low molecular weight compounds while compounds with a high molecular weight are underestimated. Regarding the differences in molecular weight between the studied fragrance substances, a variation is seen if the ranking list of the sensitisation potency is based on EC3 (%) or EC3 (M) since some substances have a molecular weight twice as high as others.

From comparisons in Table 8-1, we notice that, e.g. hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) has an EC3 value of 17.1 %, or 0.81 M when the calculation includes its molecular weight, while for trans-2-hexenal the corresponding values are 2.6% and 0.26 M. The example shows that comparing the sensitisation potency between these two substances using the EC3 values in % exaggerates the sensitisation potency of trans-2-hexenal compared to that of HICC. When using the EC3 values in molar concentrations the difference is not so pronounced.

### 8.3. Summary of animal data by LLNA

The distribution of sensitising potency of fragrance substances compared to other substances, (e.g. biocides, dyes, plastic materials) taken from three references (164-166) as assessed by EC3 values in the LLNA, is shown in Figure 8-1 and Table 8-3.

For 10 substances, no EC3-value could be established. These should have been tested at higher concentrations – some of these would most probably have generated an EC3 value. However, we reported here "No EC3 value established". 5 substances should have been tested also at lower concentration and in these cases the EC3 value could have been lowered, meaning a more severe potency category could have been achieved. In all, approx 150 experiments were reported in (164), listed in Annex II.

The median EC3 value of evaluable fragrance substances (5.9%) is similar to other substances tested (5.5%). However, very few fragrance substances have low EC3 values (≤ 2).

Substances with an EC3 value ≤2 may be categorised as strong or extreme sensitisers. Such potent sensitisers are comparatively rare among fragrance substances assessed in the LLNA. Nevertheless, fragrances are important allergens in humans, which points to repeated skin exposure to less potent sensitisers as a factor strongly determining sensitisation risk.

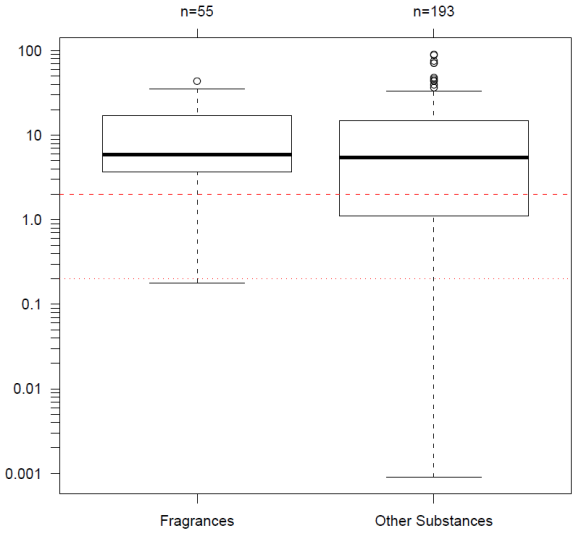


**Figure 8-1:** The distribution of fragrance chemicals and a variety of other chemicals (e.g. biocides, dyes, plastic materials), taken from the three references (164-166), are depicted as boxplots on a logarithmic scale. The bottom of the box denotes the 1st quartile (25% percentile), the thick line in the box the median, and the top of the box the 3rd quartile (75% percentile). Outliers, i.e. below the 25% and above the 75% percentiles, are shown as whiskers. Beyond the 1.5-fold interquartile range, single values are shown as circles instead of whiskers. The difference in distribution is not significant (Wilcoxon test: p=0.061).

Note: EC3 values for the five oxidised fragrances additionally examined (Table 8-2) range from 3.0 to 4.8 (median 4.4) and are lower by a factor of around 7 than EC3 values of the respective non-oxidised material.

**Table 8-3:** Summary of EC3 values for fragrance substances in Table 8-1 and for other substances, all taken from the three references (164-166). The EC3 value intervals for potency categorisation (161, 205) were used for comparison of fragrances substances vs other substances.

| **EC3 value interval** | **Fragrance substances** | | **Other substances** | |
|---|---|---|---|---|
| | **n** | **%** | **n** | **%** |
| ≤ 0.2 | 2 | 3% | 28 | 11% |
| > 0.2 - ≤ 2 | 3 | 4% | 38 | 15% |
| > 2 | 50 | 71% | 127 | 49% |
| No EC3 value established * | 10 | 14% | 0 | 0% |
| No EC3 value calculated (NC) | 5 | 7% | 69 | 26% |
| **All substances** | **70** | | **262** | |

Note: * Substances should have been tested also at higher concentrations.

## 8.4. Conclusions

- In the event that human data are lacking, the LLNA provides important information on skin sensitising potential and potency.
- Animal data on fragrance substances submitted by IFRA (164) and assessed in this opinion were generated exclusively by LLNA. Other guideline methods are, however, also available.
- The vast majority of the submitted (164) and additional (165, 166) fragrance substances tested by the LLNA are skin sensitisers.
- Several studies in the IFRA report (164) were of insufficient quality, not following the OECD guideline.

- Fragrance substances that can be predicted to autoxidise upon air exposure should also be tested after air exposure, as oxidation may significantly increase their sensitising potency.
- It can be concluded that the skin sensitising potency, as assessed by the LLNA, is only one of several factors that are of importance for sensitisation to fragrance substances. This is illustrated by the fact that only a small fraction of sensitising fragrance substances can be categorised as an extreme allergen based on LLNA test results. Therefore, doses from repeated deposition onto skin must be considered a driving force of sensitisation risk.

## 9. Structure activity relationships (SAR): grouping of substances based on expert judgement

Whether or not a particular chemical will be a sensitiser, and how potent it will be if it is a sensitiser, depends on its ability, either directly or after activation, to react with appropriate proteins in the skin. This fundamental concept was intitially demonstrated by Landsteiner and Jacobs in 1936 (208) and subsequently validated by numerous studies with various types of chemicals (some key references: (209-213)). The ability to predict sensitisation potency, or lack of it, depends on being able to predict reactivity to skin proteins. This is the basis of SAR analysis for skin sensitisation. The prediction can often be made based on the chemical structure, recognising structural features (referred to as **structural alerts**) that are associated with reactivity.

The relationships between molecular structure and reactivity that form the basis for structural alerts are based on well established principles of mechanistic organic chemistry (214). Examples of structural alerts are aliphatic aldehydes (alerting to the possibility of sensitisation via a Schiff base reaction with protein amino groups), and $\alpha,\beta$-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.

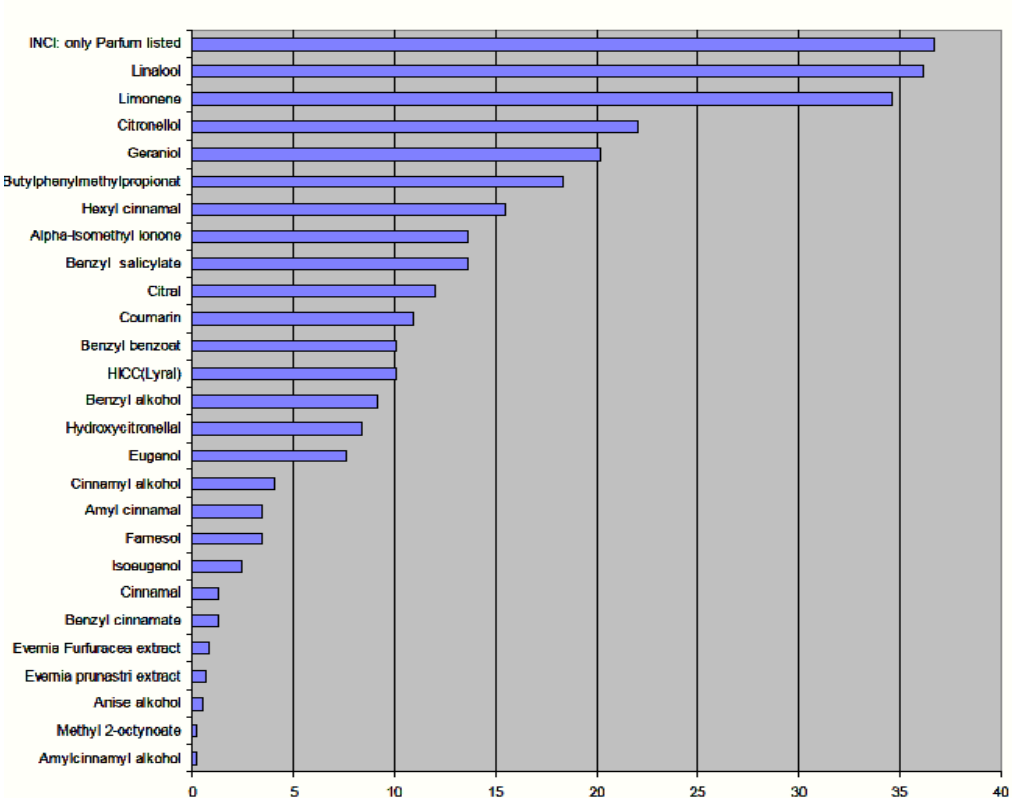


**Figure 10-1:** Frequency of occurrence in 3,000 consumer products of the 26 fragrance allergens that are required to be labelled in cosmetics and detergents (CVUA Karlsruhe, Germany, 2006/2007), according to (105).

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 |

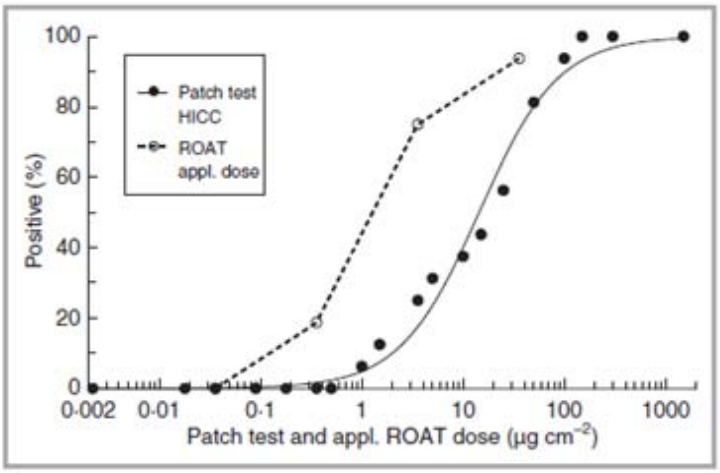

**Figure 11-1:** The fitted dose-response curve for patch test (solid line) is seen to be displaced to the right compared to the observed response from repeated open applications of the same allergen (HICC). It means that a smaller dose per application is needed to elicit a response than by one single 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.

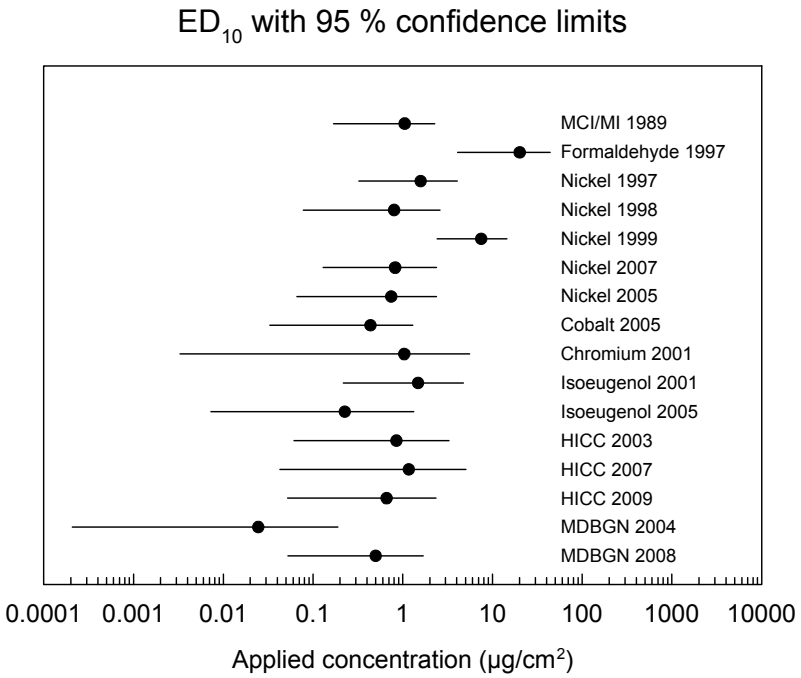


**Figure 11-2:** The threshold data with 95% confidence intervals from Table 11-6 presented graphically, after (280).

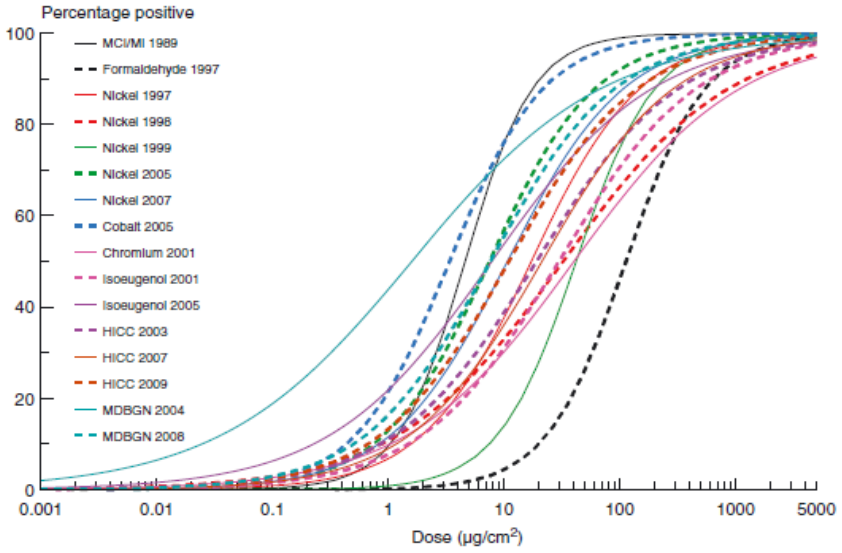


**Figure 11-3:** The fitted dose-response curves from the studies in Table 11-6**,** which are the basis for estimation of the ED10 value, after (280).

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.

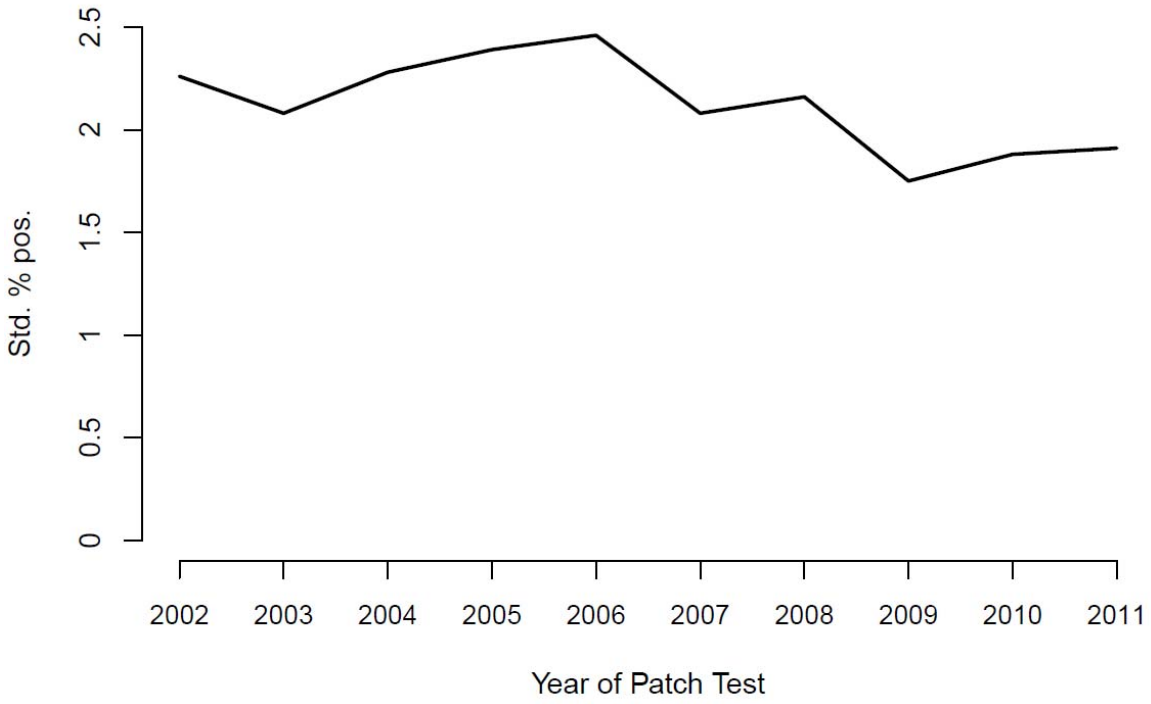


**Figure 11-4:** Time trend of hydroxyisohexyl 3-cyclohexene carboxaldehyde sensitisation prevalence [standardised prevalence of positives (%)] during 2002-2011. The decrease over time is statistically significant, after **(294)**.

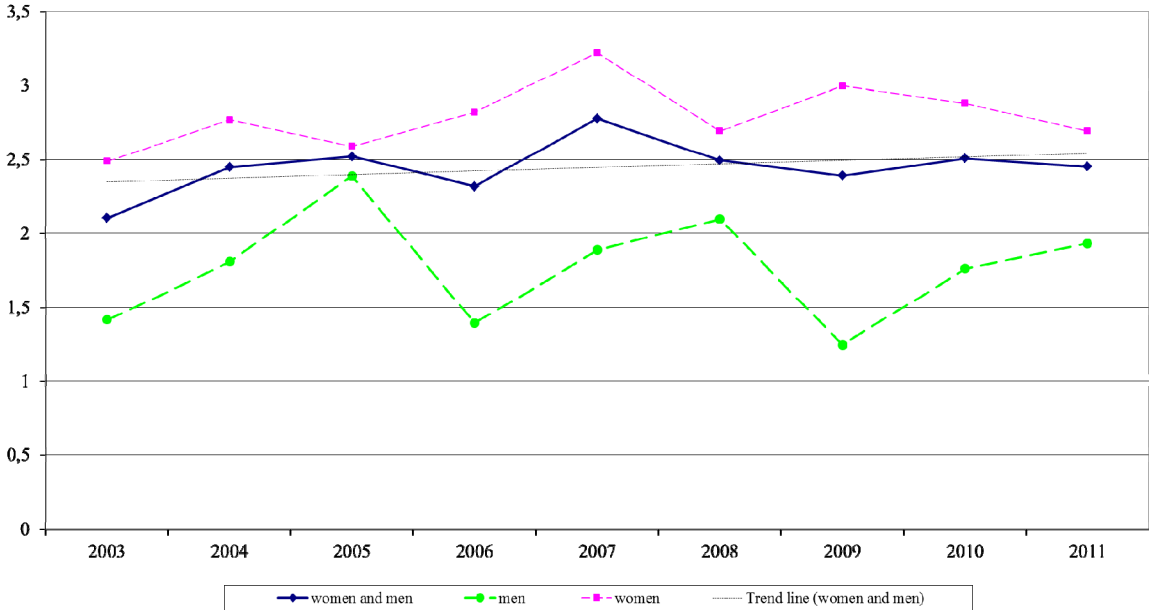


**Figure 11-5:** Prevalence of positive patch test reactions to hydroxyisohexyl 3-cyclohexene carboxaldehyde over time in 37 860 subjects tested by the Danish Contact Dermatitis Group (295).

## 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 )<br>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.);**<br>**+++ (ox.)** |
| **LINALOOL*** | **78-70-6** | **++ (non-ox.)**<br>**+++ (ox.)** |
| LINALYL ACETATE | 115-95-7 | + (non-ox.)<br>++ (ox.) |
| MENTHOL | 1490-04-6 / 89-78-1 / 2216-51-5 | ++ |
| 6-METHYL COUMARIN | 92-48-8 | ++ |
| METHYL 2-OCTYNOATE* | 111-12-6 | ++ |
| METHYL SALICYLATE | 119-36-8 | + |
| 3-METHYL-5-(2,2,3-TRIMETHYL-3- | 67801-20-1 | ++ (r.t.) |

| INCI name (or, if none exists, perfuming name according to CosIng) | CAS number | Human evidence: see text |
|---|---|---|
| CYCLOPENTENYL)PENT-4-EN-2-OL | | |
| alpha-PINENE and beta-PINENE | 80-56-8 and 127-91-3, resp. | ++ |
| PROPYLIDENE PHTHALIDE | 17369-59-4 | + (r.t.) |
| SALICYLALDEHYDE | 90-02-8 | ++ |
| alpha-SANTALOL and beta-SANTALOL | 115-71-9 and 77-42-9, resp. | ++ |
| SCLAREOL | 515-03-7 | + |
| TERPINEOL (mixture of isomers) | 8000-41-7 | + |
| alpha-TERPINEOL | 10482-56-1 / 98-55-5 | |
| Terpinolene | 586-62-9 | + |
| TETRAMETHYL ACETYLOCTAHYDRONAPHTHALENES | 54464-57-2 / 54464-59-4 / 68155-66-8 / 68155-67-9 | + |
| TRIMETHYL-BENZENEPROPANOL (Majantol) | 103694-68-4 | ++ |
| VANILLIN | 121-33-5 | ++ |
| **Natural extracts** | | |
| ***CANANGA ODORATA and Ylang-ylang oil*** | **83863-30-3; 8006-81-3** | **+++** |
| *CEDRUS ATLANTICA BARK OIL* | 92201-55-3; 8000-27-9 | ++ |
| *CINNAMOMUM CASSIA LEAF OIL CINNAMOMUM ZEYLANICUM BARK OIL* | 8007-80-5 84649-98-9 | ++ (r.t.) |
| *CITRUS AURANTIUM AMARA FLOWER / PEEL OIL* | 8016-38-4; 72968-50-4 | ++ |
| *CITRUS BERGAMIA PEEL OIL EXPRESSED* | 89957-91-5 | + (r.t.) |
| *CITRUS LIMONUM PEEL OIL EXPRESSED* | 84929-31-7 | ++ |
| *CITRUS SINENSIS (syn.: AURANTIUM DULCIS) PEEL OIL EXPRESSED* | 97766-30-8; 8028-48-6 | ++ |
| *CYMBOPOGON CITRATUS / SCHOENANTHUS OILS* | 89998-14-1; 8007-02-1; 89998-16-3 | ++ |
| *EUCALYPTUS SPP. LEAF OIL* | 92502-70-0; 8000-48-4 | ++ |
| ***EUGENIA CARYOPHYLLUS LEAF / FLOWER OIL*** | **8000-34-8** | **+++** |
| ***EVERNIA FURFURACEA EXTRACT**** | **90028-67-4** | **+++** |
| ***EVERNIA PRUNASTRI EXTRACT**** | **90028-68-5** | **+++** |
| ***JASMINUM GRANDIFLORUM / OFFICINALE*** | **84776-64-7; 90045-94-6; 8022-96-6** | **+++** |
| *JUNIPERUS VIRGINIANA* | 8000-27-9; | ++ |

| INCI name (or, if none exists, perfuming name according to CosIng) | CAS number | Human evidence: see text |
|---|---|---|
| | 85085-41-2 | |
| *LAURUS NOBILIS* | 8002-41-3; 8007-48-5; 84603-73-6 | ++ |
| *LAVANDULA HYBRIDA* | 91722-69-9 | + (r.t.) |
| *LAVANDULA OFFICINALIS* | 84776-65-8 | ++ |
| *MENTHA PIPERITA* | 8006-90-4; 84082-70-2 | ++ |
| *MENTHA SPICATA* | 84696-51-5 | ++ |
| ***MYROXYLON PEREIRAE*** | **8007-00-9;** | **++++** |
| *NARCISSUS SPP.* | diverse | ++ |
| *PELARGONIUM GRAVEOLENS* | 90082-51-2; 8000-46-2 | ++ |
| *PINUS MUGO/PUMILA* | 90082-72-7 / 97676-05-6 | ++ |
| *POGOSTEMON CABLIN* | 8014-09-3; 84238-39-1 | ++ |
| *ROSE FLOWER OIL (ROSA SPP.)* | Diverse | ++ |
| ***SANTALUM ALBUM*** | **84787-70-2; 8006-87-9** | **+++** |
| ***TURPENTINE (oil)*** | **8006-64-2; 9005-90-7; 8052-14-0** | **++++** |
| **VERBENA ABSOLUTE** | 8024-12-2 | ++ |

**Table 13-2:** Fragrance substances categorised as established contact allergens in animals.

| INCI name (or, if none exists, perfuming name according to CosIng) | CAS number | Human evidence: see text | EC 3 value (min; %) |
|---|---|---|---|
| **Individual chemicals** | | | |
| Allyl phenoxyacetate | 7493-74-5 | none | 3.1 |
| p-tert. -Butyldihydrocinnamaldehyde | 18127-01-0 | none | 4.3 |
| CYCLAMEN ALDEHYDE | 103-95-7 | none | 22 |
| Dibenzyl ether | 103-50-4 | none | 6.3 |
| 2,3-DIHYDRO-2,2,6-TRIMETHYLBENZALDEHYDE | 116-26-7 | limited | 7.5 |
| trans-2-Hexenal | 6728-26-3 | none | 2.6 |
| 2-Hexylidene cyclopentanone | 17373-89-6 | none | 2.4 |
| HEXYL SALICYLATE | 6259-76-3 | negative | 0.18 |
| p-Isobutyl-α-methyl hydrocinnamaldehdye | 6658-48-6 | none | 9.5 |

| INCI name (or, if none exists, perfuming name according to CosIng) | CAS number | Human evidence: see text | EC 3 value (min; %) |
|---|---|---|---|
| Isocyclocitral | 1335-66-6 | none | 7.3 |
| α-Methyl cinnamic aldehyde | 101-39-3 | none | 4.5 |
| METHYLENEDIOXYPHENYL METHYLPROPANAL | 1205-17-0 | none | 16.4 |
| METHYLUNDECANAL | 110-41-8 | none | 10 |
| 2-Methoxy-4-methylphenol | 93-51-6 | none | 5.8 |
| 4-Methoxy-α-methyl benzenpropanal | 5462-06-6 | none | 23.6 |
| METHYL OCTINE CARBONATE | 111-80-8 | limited | 2.5 |
| Perillaldehyde p-Mentha-1,8-dien-7-al | 2111-75-3 | none | 8.1 |
| PHENYLACETALDEHYDE | 122-78-1 | limited | 3 |
| **Natural extracts** | | | |
| Jasminum Sambac Flower CERA / Extract / Water | 91770-14-8 | none | 35.4 |

**Table 13-3:** Fragrance substances categorised as likely contact allergens by combination of evidence.

| INCI name (or, if none exists, perfuming name according to CosIng) | CAS number | Human evidence: see text | EC 3 value (min; %) | SAR |
|---|---|---|---|---|
| AMBRETTOLIDE | 7779-50-2 | limited | none | + |
| CARVACROL | 499-75-2 | limited | none | + |
| Citrus paradisi § | 8016-20-4 | none | R43 | n.a. |
| CUMINALDEHYDE | 122-03-2 | limited | none | + |
| CYCLOPENTADECANONE | 502-72-7 | limited | none | + |
| trans-trans-delta-DAMASCONE | 71048-82-3 | limited | none | + |
| 2,4-dimethyl-3-cyclohexen-1-carboxaldehyde § | 68039-49-6 | none | R43 | + |
| DIMETHYLTETRAHYDRO BENZALDEHYDE | 68737-61-1 | limited | none | + |
| ETHYL VANILLIN | 121-32-4 | limited | none | + |
| HELIOTROPINE | 120-57-0 | limited | none | + |
| ISOAMYL SALICYLATE | 87-20-7 | limited | none | ++ |
| ISOLONGIFOLENEKETONE | 33407-62-4 | limited | none | + |
| Longifolene § | 475-20-7 | none | R43 | + |
| Mentha arvensis § | 68917-18-0 | none | R43 | n.a. |
| METHOXYCITRONELLAL | 3613-30-7 | limited | none | + |
| METHYL CINNAMATE | 103-26-4 | limited | none | ++ |
| METHYLIONANTHEME | 55599-63-8 | limited | none | + |

| INCI name (or, if none exists, perfuming name according to CosIng) | CAS number | Human evidence: see text | EC 3 value (min; %) | SAR |
|---|---|---|---|---|
| 5-METHYL-alpha-IONONE | 79-69-6 | limited | none | + |
| MYRCENE | 123-35-3 | limited | none | ++ |
| MYRTENOL | 515-00-4 | limited | none | + |
| NEROL | 106-25-2 | limited | none | ++ |
| Nerolidol (isomer not specified) | 7212-44-4 | limited | none | ++ |
| NOPYL ACETATE | 128-51-8 | limited | none | + |
| PHYTOL | 150-86-7 | limited | none | + |
| RHODINOL | 6812-78-8 | limited | none | + |
| trans-ROSE KETONE-5 | 39872-57-6 | limited | none | ++ |

§ Substances/natural mixtures were classified as R43, according to the submission by IFRA. The evidence on which this classification was based was not available to the SCCS, so the validity of classification cannot be assessed. Nevertheless, the four substances/substance mixtures should be treated as *likely contact allergens*.

n.a.: not applicable (natural mixture)

**Table 13-4:** Fragrance substances categorised as possible contact allergens.

| INCI name (or, if none exists, perfuming name according to CosIng) | CAS number | Human evidence: see text | EC 3 value (min; %) | SAR |
|---|---|---|---|---|
| **Individual chemicals** | | | | |
| CYCLOHEXYL ACETATE | 622-45-7 | limited | none | 0 |
| ETHYLENE DODECANEDIOATE | 54982-83-1 | limited | none | 0 |
| HYDROXYCITRONELLOL | 107-74-4 | limited | none | 0 |
| METHOXYTRIMETHYLHEPTANOL | 41890-92-0 | limited | none | 0 |
| METHYL p-ANISATE | 121-98-2 | limited | none | 0 |
| METHYL DIHYDROJASMONATE | 24851-98-7 | limited | none | 0 |
| PHENETHYL ALCOHOL | 60-12-8 | limited | none | 0 |
| PHENYLPROPANOL | 122-97-4 | limited | none | 0 |
| AMYLCYCLOPENTANONE | 4819-67-4 | negative | none | + |
| BENZYL ACETATE | 140-11-4 | negative | none | + |
| 6-ETHYLIDENEOCTAHYDRO-5,8-METHANO-2H-BENZO-1-PYRAN | 93939-86-7 | negative | none | + |
| 3a,4,5,6,7,7a-HEXAHYDRO-4,7-METHANO-1H-INDEN-5(OR 6)-YL ACETATE | 54830-99-8 | negative | none | + |
| alpha-IONONE | 127-41-3 | negative | none | + |
| beta-IONONE | 79-77-6 | negative | none | + |
| METHYL IONONE (mixture of | 1335-46-2 | negative | none | + |

| INCI name (or, if none exists, perfuming name according to CosIng) | CAS number | Human evidence: see text | EC 3 value (min; %) | SAR |
|---|---|---|---|---|
| isomers) | | | | |
| TERPINEOL ACETATE (Isomer mixture) | 8007-35-0 | negative | none | + |
| alpha-TERPINYL ACETATE | 80-26-2 | negative | none | + |
| CITRONELLYL NITRILE | 51566-62-2 | none | none | ++ |
| alpha-CYCLOHEXYLIDENE BENZENEACETONITRILE | 10461-98-0 | none | none | + |
| DECANAL | 112-31-2 | none | none | ++ |
| DIHYDROMYRCENOL | 18479-58-8 | none | none | + |
| 3,7-DIMETHYL-1,6-NONADIEN-3-OL | 10339-55-6 | none | none | ++ |
| 2-ETHYL-4-(2,2,3-TRIMETHYL-3-CYCLOPENTEN-1-YL)-2-BUTEN-1-OL | 28219-61-6 | none | none | + |
| GERANYL ACETATE | 105-87-3 | none | none | ++ |
| HEXAHYDRO-METHANOINDENYL PROPIONATE | 68912-13-0 | none | none | + |
| IONONE isomeric mixture | 8013-90-9 | none | none | + |
| ISOBERGAMATE | 68683-20-5 | none | none | + |
| METHYL DECENOL | 81782-77-6 | none | none | + |
| TRICYCLODECENYL PROPIONATE | 17511-60-3 | none | none | + |
| OXACYCLOHEXADECENONE | 34902-57-3 | none | none | ++ |
| VERDYL ACETATE | 2500-83-6/ 5413-60-5 | none | none | + |
| trans-beta-Damascone | 23726-91-2 | none | none | + |
| gamma-Damascone | 35087-49-1 | none | none | + |
| Citronellal | 106-23-0 | none | none | ++ |
| Phenethyl salicylate | 87-22-9 | none | none | ++ |
| **Natural extracts** | | | | |
| ACORUS CALAMUS ROOT OIL | 84775-39-3 | Limited | none | |
| CEDRUS DEODARA WOOD OIL | 91771-47-0 | Limited | none | |
| CITRUS AURANTIUM AMARA LEAF OIL | 72968-50-4 | Limited | none | |
| CITRUS TANGERINA … | 223748-44-5 | Limited | none | |
| CYMBOPOGON NARDUS / WINTERIANUS HERB OIL | 89998-15-2; 91771-61-8 | Limited | none | |
| ILLICIUM VERUM FRUIT OIL | 84650-59-9 | Limited | none | |
| LAVANDULA SPICA | 97722-12-8 | Limited | none | |

| INCI name (or, if none exists, perfuming name according to CosIng) | CAS number | Human evidence: see text | EC 3 value (min; %) | SAR |
|---|---|---|---|---|
| LITSEA CUBEBA | 90063-59-5 | Limited | none | |
| PELARGONIUM ROSEUM | 90082-55-6 | Limited | none | |
| SALVIA spp. | Diverse | Limited | none | |
| TAGETES PATULA | 91722-29-1 | Limited | none | |
| THYMUS spp. | 84929-51-1 | Limited | none | |
| VETIVERIA ZIZANOIDES | 8016-96-4; 84238-29-9 | Limited | none | |

Regarding the above categorisation of fragrance substances, the following aspects need to be considered when interpreting an outcome other than established contact allergen in humans:

- If human evidence is negative, there is still a potential sensitisation risk, as in this set of substances the number of (consecutive) patients tested was low, i.e. up to a few hundred.
- If EC3 values are given as higher (>) than a certain value (see 8.3), an exact EC3 could not be established, as the substance had been tested in too low concentration(s). In these cases, the substances have not been categorised as 'established contact allergen in animals'.
- For SAR, the categories of prediction are: non-sensitiser (0); possible-sensitiser (+); predicted sensitiser (++); and not predictable (n.p.). (For details see Table 9-3 and Table 9-4). SAR predictions are only considered when human and animal data are limited or missing.
- Several substances are currently banned from the use in cosmetic products by Annex II of the Cosmetics Directive, based on concerns regarding one or more toxicological endpoints. While available clinical evidence regarding this set of substances is listed in Annex I to this opinion, these substances have not further been evaluated.

Fragrance ingredients listed in Table 13-1 clearly have caused disease in man, and based on the clinical experience alone, these 82 substances were classified as established contact allergens in humans, 54 individual chemicals and 28 natural extracts (mixtures of chemicals), including all 26 fragrance allergens identified by SCCNFP in 1999. For a number of other substances, no patch test data were available, but positive animal data, obtained by a validated guideline method (LLNA) addressing hazard, indicate that a – yet not quantified – risk for humans is very likely to exist, given sufficient exposure. In other cases only in a relatively small number of patients has been tested positively ('limited human evidence'). Here, combination with SAR analyses corroborates the conclusion that these substances, too, are sufficiently qualified to be regarded as 'likely fragrance allergens'.

Of those 82 substances identified as established contact allergens in humans, 12 chemicals (listed in Table 13-5) and eight natural extracts are considered of special concern as they have given rise to at least 100 reported cases. These substances pose a particularly high risk of sensitisation to the consumer and are further considered in the answer of question 2. One substance, hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), was shown to be the cause of allergic contact dermatitis in more than 1500 reported cases since 1999. The number of cases is only those reported in scientific publications, and therefore the actual number of cases is severely under-estimated.

**Table 13-5:** Established fragrance contact allergens of special concern (single chemicals only).

| |
|---|
| Cinnamal |
| Cinnamyl Alcohol* |
| Citral |
| Coumarin |
| Eugenol* |
| Farnesol* |
| Geraniol* |
| Hydroxycitronellal |
| Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) |
| Isoeugenol* |
| Limonene (oxidised) |
| Linalool* (oxidised) |

*including their respective esters

The established contact allergens in animals (Table 13-2) and the likely contact allergens, identified based on a combination of limited evidence from man together with positive SAR predictions (Table 13-3), are predicted to cause disease in man given sufficient exposure.

Information on the presence of all the substances given in Table 13-1, Table 13-2 and Table 13-3 in cosmetic products is important in order to enable aimed testing of patients with contact dermatitis and to diagnose fragrance allergy without delay. Further, this information is important to the sensitised consumer as it will enable them to avoid cosmetic products, which they may not tolerate.

Substances given in Table 13-4 are possible contact allergens and further data are required to judge if these are contact allergens in humans and give rise to contact allergy in consumers.

## Conclusions - Question 1

The studies since the SCCNFP Opinion on fragrance allergy in consumers (1) have confirmed that the fragrance allergens currently listed in Annex III, entries 67-92 are still relevant fragrance allergens for the consumers from their exposure to cosmetic products.

The review of the clinical and experimental data shows that many more fragrance substances than those identified in the SCCNFP opinion of 1999 have been shown to be sensitisers in humans. A comprehensive list of established contact allergens in humans is given in Table 13-1.

Moreover, animal experiments indicate that additional fragrance substances can be expected to be contact allergens in humans, although human evidence is currently lacking.

Additionally, limited human and/or animal evidence together with structure activity relationship analysis suggests that other fragrance ingredients may be a cause of concern with regard to their potential of causing contact allergy in humans.

Ingredient listing is important in clinical practice for the management of patients who are allergic to one or more of the listed fragrance chemicals. It is also important for the

patients in order to avoid future exposure to fragrance contact allergens which they may not tolerate.

The SCCS considers that those substances itemised in Table 13-1, Table 13-2 and Table 13-3 represent those fragrance ingredients that the consumer should be made aware of when present in cosmetic products.

Substances known to be transformed (e.g. hydrolysis of esters) to known contact allergens should be treated as equivalent to these known contact allergens. The combined concentration of the alcohol and its ester must be considered regarding exposure. Important indicative, but not exhaustive, examples include isoeugenol and its esters, geraniol and its esters, eugenol and its esters, and linalool and its esters.

### 13.2. Question 2

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

Dose-response relationships exist between exposure to contact allergens and the proportion of consumers who will become sensitised to an allergen (i.e. induction), as well as the proportion who will suffer from allergic contact dermatitis (elicitation). For a number of recognised contact allergens in man, dose-elicitation studies on sensitised individuals are available. These studies indicate that it is in principle possible to derive exposure levels that the majority of sensitised individuals will tolerate. The SCCS considers that thresholds based on elicitation levels in sensitised individuals will be sufficiently low to protect both the majority of sensitised individuals as well as most of the non-sensitised consumers from developing contact allergy and limit the risk of induction.

Among the established chemical fragrance allergens, 12 were identified as posing a high risk of sensitisation to the consumer (Table 13-5), i.e. more than 100 reported cases. For these substances, limitation of exposure would help to protect sensitised consumers from developing allergic contact dermatitis.

In cases where specific data of sufficient quality on threshold levels for a particular allergen are available, these data should be used to set an individual safe threshold. However, when such quality data are not available and a substance has been identified to pose a high risk of sensitisation to the consumer, a general threshold limit can be applied.

Dose-response studies have been performed with only four of these fragrance substances (HICC, isoeugenol, cinnamal and hydroxycitronellal). In addition, such a study has also been performed on chloroatranol, a potent allergen in *Evernia prunastri and Evernia furfuracea*. These studies, however, are not adequate to derive safe thresholds for the individual substances directly from the data.

If no adequate data are available, for substances posing a high risk to the consumer (like the 12 listed in Table 13-5), the use of a general threshold may be considered. A threshold of 0.8 μg/cm$^2$ has been derived based on a statistical analysis of the available data in the scientific literature, including two fragrance allergens. This corresponds to 0.01% (100 ppm) limit in cosmetic products indicative for safe use. This approximation may hold for weak to strong allergens. However, some strong and extreme sensitisers may require lower individual thresholds. As an example, chloroatranol, present in the natural product *Evernia prunastri* and in *Evernia furfuracea*, has been shown to have an elicitation threshold of 0.0004 μg/cm$^2$ under experimental conditions similar to those yielding above results. On the other hand, for very weak sensitisers, this generic threshold may be too conservative.

The model providing the general threshold of 100 ppm has been based on single substances only and no general safe level for the natural extracts of concern can be

identified, but the maximum use concentration applies to the identified fragrance allergens also when present in the natural extract.

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) has been the most frequently reported chemical causing fragrance allergy since the 1999 opinion on fragrance allergy. In total, reports of more than 1500 cases have been published in the scientific literature (see chapter 7.1 and Annex I), which will severely underestimate the actual prevalence in the population. HICC has been shown to be a significant cause of disease as many of those with contact allergy to HICC had also reactions to cosmetics, which contained or were likely to contain HICC. The SCCP concluded in 2003 that 200 ppm of HICC would be tolerated by the majority of sensitised individuals and this level of exposure would have a low potential to induce sensitisation (241). Since 2003 attempts have been made by the fragrance industry to contain the outbreak of HICC allergy, but with no convincing success so far. Recent voluntary restrictions (recommendations to lower use concentrations, at least for some product types, to the level recommended by the SCCS in 2003) are not reflected in available evidence and are considered insufficient. The SCCS considers that the number of cases of HICC allergy documented over the last decade is exceptionally high and that continued exposure to HICC by the consumer is not considered safe, even at concentrations as low as 200 ppm. Chloroatranol and atranol are the main allergenic components of *Evernia prunastri* and *Evernia furfuracea*. The SCCS concluded in 2004 (239) that these should not be present in cosmetic products, due to their exceptionally high sensitisation potential. Attempts to effectively reduce the content of these compounds in "oak moss abs." (300) have largely failed to reduce contact allergy to *Evernia prunastri* and *Evernia furfuracea* and the data presented in this opinion show that the number of cases remains high.

### Conclusions - Question 2

There are two components to the safety of fragrance ingredients in terms of contact allergy. First, the need to eliminate or reduce induction of contact allergy (primary prevention), which, when it occurs, is life long. Secondly, the need to eliminate or reduce elicitation reactions (secondary prevention) on the skin of those individuals who are already sensitised. Human dose elicitation experiments have hithereto been performed only for a very small number of substances. It is unlikely that more of these studies will be performed due to experimental and subject recruitment difficulties.

For individual substances, no levels that could be considered safe for the majority of consumers could be established from the available data.

The dose elicitation studies available indicate that a general level of exposure of up to 0.8 $\mu g/cm^2$ (0.01%) may be tolerated by most consumers with contact allergy to fragrance allergens. The SCCS considers that this level of exposure could be efficient in limiting elicitation unless there is substance specific data, either experimental or clinical, to the contrary.

Such a threshold based on elicitation levels in sensitised individuals will be sufficiently low to protect both sensitised individuals as well as most of the non-sensitised consumers from developing contact allergy.

The SCCS is of the opinion that for substances idendified as posing a high risk to the consumer and for which no individual thresholds could be derived (Table 13-5), the general threshold of 0.01% would limit the problem of fragrance allergy in the consumer significantly.

It was not possible to provide a safe threshold for natural extracts of concern, as no specific investigations exist and the model providing the general threshold (0.01%) has been based on individual chemicals only. However the SCCS considers that the maximum use concentration applies to the above identified fragrance allergens also when present in the natural extract. This will also reduce the risk of sensitisation and elicitation from natural extracts.

It is important to stress that this general threshold, although limiting the problem, does not preclude that the most sensitive segment of the population may react upon exposure to these levels. Hence, this threshold does not remove the necessity for providing information to the consumer concerning the presence of the fragrance substance in cosmetics.

In the case of hydroxyisohexyl 3-cyclohexene carboxaldehyde, in 2003 the SCCP suggested that levels of up to 200 ppm would be tolerated by the majority of sensitised individuals. Recent voluntary restrictions (recommendations to lower use concentrations, at least for some product types, to the level recommended by the SCCS in 2003) are not reflected in available evidence and are considered insufficient. The SCCS considers that the number of cases of HICC allergy documented over the last decade is exceptionally high and that continued exposure to HICC by the consumer is not considered safe, even at concentrations as low as 200 ppm. Therefore, HICC should not be used in consumer products in order to prevent further cases of contact allergy to HICC and to limit the consequences to those who already have become sensitized. The SCCP concluded in 2004 that chloroatranol and atranol, the main allergenic constituents of *Evernia prunastri and Evernia furfuracea*, should not be present in products for the consumer. The persistently high frequency of contact allergy to *Evernia prunastri* and *Evernia furfuracea* noted in eczema patients does point to a persisting problem with exposure to allergenic constituents, despite efforts to reduce the allergen content (296). The SCCS is of the opinion that the presence of the two constituents, chloroatranol and atranol, in cosmetic products are not safe.

### 13.3. Question 3

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

Many fragrance substances can act as prehaptens or prohaptens, forming allergens which are more potent than the parent substance by abiotic and/or metabolic activation, and thus increasing the risk of sensitisation.

Experimental and clinical studies have shown that there are fragrance substances that act as prehaptens, i.e. their sensisitation potency is markedly increased by air exposure due to oxidation (autoxidation). Non/low-sensitising compounds are thereby transformed into more potent sensitisers. Limonene, linalool, linalyl acetate, alpha-terpinene and geraniol have all been identified as prehaptens. These fragrance substances are common in scented cosmetics as well as in household products. The clinical studies show that the exposure to allergens formed due to autoxidation causes significant contact allergy in consumers. Patch testing with oxidised limonene and oxidised linalool shows that these substances rank among the most common contact allergens.

In the SAR analyses performed in this work by the SCCS, fragrance compounds with structural alerts that indicate that they are possible prehaptens have been identified (Table 9-1, Table 9-2). In such cases further thorough investigations are needed. It is also important to investigate the stability of the primary oxidation products (the hydroperoxides) formed from various structures of fragrance compounds. The stability of these compounds can have great impact on the sensitisation potency of the oxidised compound as they are strong sensitisers. However, the secondary oxidation products (aldehydes and epoxides) can also be important sensitisers depending on the overall structure of the compound as was demonstrated for oxidised geraniol.

Air oxidation of prehaptens can be prevented to a certain extent by measures during handling and storage of the ingredients and final products to avoid air exposure, and/or by addition of suitable antioxidants. The autoxidation rate depends not only on the compound itself, but also on its purity. The prevention of autoxidation using antioxidants

needs thorough investigation because antioxidants can exert their function by being oxidised instead of the compound that they protect and might thereby be activated to skin sensitising derivatives after oxidation. As antioxidants are now frequently used at elevated concentrations in scented products due to a growing awareness of the problem of autoxidation, there is a risk that sensitisation caused by the antioxidants will rise. One of the most used antioxidants is butylated hydroxytoluene (BHT) which is considered a minimal risk for sensitisation in the concentrations used but nevertheless, with increased concentrations and usage, the risk of sensitisation could increase.

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

Based on the clinical data, oxidised limonene and oxidised linalool are allergens of high concern (Table 13-5) which pose a high risk of sensitisation to the consumer. For these substances the presence of the oxidised fraction represented by the peroxide content should not be higher than 10 ppm. Alternatively, the suggested general threshold dose/area of 0.8 μg/cm$^2$ (100 ppm in cosmetic products) could be applicable to the total oxidised fraction, i.e. not only peroxides but also secondary oxidation products such as aldehydes and epoxides.

Compounds that are bioactivated by metabolising enzymes to haptens are referred to as prohaptens. Established prohaptens of clinical importance are cinnamyl alcohol, geranial, geraniol, eugenol, isoeugenol and alpha-terpinene.

**Table 13-6:** Known prehaptens and prohaptens.

| Fragrance substance | Activation by air oxidation | Bioactivation (oxidation) | Bioactivation (hydrolysis) |
|---|---|---|---|
| Cinnamyl alcohol | | x | |
| Eugenol | | x | |
| Eugenyl acetate | | x | x |
| Geranial | x | x | |
| Geraniol | x | x | |
| Geranyl acetate | x | x | x |
| Isoeugenol | | x | |
| Isoeugenyl acetate | | x | x |
| Limonene | x | | |
| Linalool | x | | |
| Linalyl acetate | x | | |
| alpha-Terpinene. | x | x | |

When bioactivation occurs, the risk of cross-reactivity should be considered. An increased complexity in the cross-reactivity pattern is obtained when a compound could act both as a prehapten and a prophapten. For instance, it is known that cinnamyl alcohol and cinnamal can cross-react due to the formation of common sensitising substances. The same applies to geraniol and citral.

In case derivatives of a fragrance substance are used, it must be taken into account that the derivative could be transformed into the parent or a cross-reacting compound. For such derivatives the same rules as for the corresponding parents should apply, unless the

stability of the derivative has been demonstrated. In particular, hydrolysis of esters to the corresponding alcohols can cause cross-reactions. Acetate esters of eugenol, isoeugenol and geraniol are frequently used in cosmetics.

To be able to predict the sensitisation potency of prohaptens, steps of bioactivation have to be included in the predictive tests.

Activation of individual compounds to various haptens increases the risks of cross-reactivity between chemicals and also causes difficulties in prediction of these risks. Prediction of risks requires sound application of theoretical principles in combination with well designed experimental studies. Based on the acquired knowledge, qualified suggestions using structure activity relationship (SAR) regarding many fragrance substances have been made (Table 9-1 to Table 9-3). However, as the stability of formed oxidation products (mainly hydroperoxides) is important for the sensitisation potency, the SAR hypotheses must be followed by experimental investigations for the actual compounds.

### Conclusions - Question 3

Many fragrance substances can act as prehaptens or prohaptens, forming allergens which are more potent than the parent substance by abiotic and/or metabolic activation. Activation can thus increase the risk of sensitisation. Fragrances with published data showing the formation of sensitising compounds by autoxidation, bioactivation or both include the following (see also Table 13-6).

Fragrance substances of clinical importance known to be prehaptens and to form sensitising compounds by air oxidation are limonene, linalool, and linalyl acetate.

Fragrance substances of clinical importance known to be prohaptens and to form sensitising compounds by metabolic transformation are cinnamyl alcohol, eugenol, isoeugenol and isoeugenyl acetate.

Fragrance substances of clinical importance with published data known to be both prehaptens and prohaptens and to form sensitising compounds by air oxidation (prehaptens) and by metabolic transformation are geraniol and alpha -terpinene.

A fragrance substance that sensitises without activation but forms more potent sensitising compounds by air oxidation and also by metabolic transformation is geranial (one isomer of citral).

In the case of prehaptens, it is possible to prevent activation outside the body to a certain extent by different measures, e.g. prevention of air exposure during handling and storage of the ingredients and the final product and by the addition of suitable antioxidants. When antioxidants are used, care should be taken that they will not be activated themselves and thereby form new sensitisers.

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

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

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

The substances presented above are based on current knowledge and should be seen as indicative and illustrative of the general problem. As substances with structural alerts for acting as pro- and/or prehaptens are quite common among the fragrance substances listed (see Tables 9-1 and 9-2), the possibility for activation to generate new potent allergens should be considered.

The SCCS is of the opinion that substances known to be transformed (e.g. by oxidation either via air oxidation or via bioactivation) to known contact allergens should be treated as equivalent to these contact allergens, i.e the same restrictions and other regulatory requirements should apply, unless specific data exist that allow for an individual assessment. Important indicative examples include limonene, linalool, linalyl acetate, geraniol, geranial, alpha-terpinene, eugenol, isoeugenol and cinnamyl alcohol.

## 14. List of abbreviations

| | |
|---|---|
| ACD | Allergic contact dermatitis |
| alc. | Alcohol (as vehicle) |
| CI | Confidence interval |
| CLP | Classification, labelling and packaging |
| coloph. | Colophonium |
| DCs | Dendritic cells |
| EC | European Commission |
| ESSCA | European Surveillance System on Contact Allergies |
| EDT | Eau de toilette |
| EDP | Eau de perfume |
| EU | European Union |
| FM | Fragrance mix |
| GC | Gas chromatography |
| GPMT | Guinea pig maximisation test |
| HICC | Hydroxyisohexyl 3-cyclohexene carboxaldehyde |
| HRIPT | Human repeat insult patch test |
| IFRA | International Fragrance Association (www.ifraorg.org) |
| IVDK | Information Network of Departments of Dermatology (www.ivdk.gwdg.de) |
| INCI | International Nomenclature on Cosmetic Ingredients |
| LCs | Langerhans cells |
| LLNA | Local lymph node assay |
| MPR | *Myroxylon pereirae* resin |
| NACDG | North American Contact Dermatitis Group |
| OECD | Organization of Economic Co-operation and Development |
| ox. | oxidised |
| pet. | Petrolatum (as vehicle) |
| ppm | parts per million (10000 ppm = 1%) |
| PPV | Positive predictive value |
| PR | Prevalence ratio |
| PT(ed)(ing) | Patch test(ed) (ing) |
| QMM | Quantitative mechanistic model |
| QRA | Quantitative risk assessment |
| (Q)SAR | (Quantitative) structure activity relationship |
| REACH | Registration, Evaluation, Authorisation and restriction of CHemicals |
| RIFM | Research Institute for Fragrance Materials (www.**rifm**.org/) |

| | |
|---|---|
| ROAT | Repeated open application test |
| SC | Single constituents (of one of the fragrance mixes) |
| SCCS | Scientific Committee on Consumer Safety |
| SCCNFP | Scientific Committee on Cosmetic Products and Non-Food Products |
| SCCP | Scientific Committe on Consumer Products |
| UK | United Kingdom |
| US(A) | United States (of America) |
| UV | Ultraviolet |

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## Annex I - Catalgoue of fragrance allergens

# Contents

Single chemicals ..... 142
Catalogue of single chemicals evaluated ..... 146
Natural extracts / essential oils ..... 237
Catalogue of natural extracts / essential oils evaluated ..... 238
References ..... 277

### 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 | Geraniol | 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<br>n=320 | patients selected according to history or site suspicious of contact allergy to fragrance ingredients |

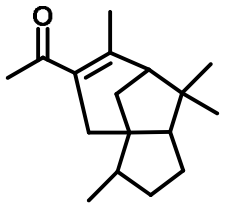
| | | | |
|---|---|---|---|
| IVDK 2010b (11) | Germany, Switzerland and one centre in Austria | 2005-2008<br>n=35633 tested with FM II, n=2217 with all SC | |

**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<br>n=1825 consecutive patients |
| An 2005 (13) | South Korea (multicentre) | 04/2002 – 06/2003<br>n=422 consecutive patients |
| Sugiura 2000 (14) | Nagoya, Japan | 1990-1998<br>n=1483 patients with suspected cosmetic dermatitis |
| Frosch 1995 (15) | 11 European depts. | Prior to 1995<br>n=1069 consecutive patients |
| Frosch 2002 a (16) | 6 European depts. | 10/1997-10/1998<br>n=1855 consecutive patients |
| Frosch 2002 b (17) | 6 European depts. | Prior to 2002<br>n=1606 consecutive patients |
| Coimbra 2000 (9) | Portugal | 07/1989-06/1999<br>n=226 with FM I SC<br>n=67 also with other fragrances |
| Larsen 1977 (18) | US | 1977<br>n=20 "perfume-sensitive patients" |
| Larsen 2001 (19) | worldwide multicentre | ? (prior to 2001)<br>n=178 patients with known contact allergy to fragrance ingredients |
| Belsito 2006 (20) | North American (5 US, 1 Canadian) depts. | 2003<br>n=1603 patients |
| NACDG 2009 (21) | US and Canada | 2005-2006<br>n= 4454 patients |
| Wöhrl 2001 (22) | "FAZ" clinic Vienna | 1997-2000<br>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<br>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 |

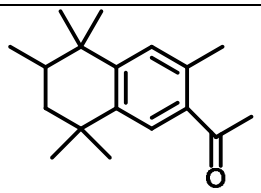
## Catalogue of single chemicals evaluated

| ***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<br>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".

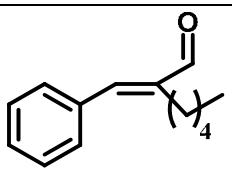
Additional information:
Acetyl cedrene (Vertofix®, IFF) is a complex mixture obtained from cedar wood oil by the acetylation of terpenes. The principal component of acetyl cedrene is methyl cedryl ketone (CAS 32388-55-9). It is a "top 100" substance (IFRA, pers. comm.2010)

| ***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).

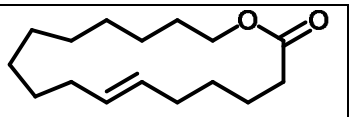
| ***AMYL CINNAMAL*** |  |
|---|---|
| CAS # 122-40-7 | |
| EC # 204-541-5 | |
| **2-(Phenylmethylene)-heptanal** | |
| Cinnamaldehyde, α-amyl- (4CI); Cinnamaldehyde, α-pentyl- (6CI,7CI,8CI); 2-(Phenylmethylene)heptanal; 2-Benzylideneheptanal; Amylcinnamaldehyde; Amylcinnamic acid aldehyde; Amylcinnamic 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).

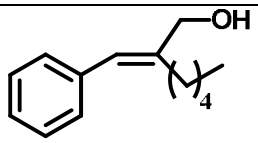
| ***AMBRETTOLIDE*** |  |
|---|---|
| CAS # 7779-50-2 | |
| EC # 231-929-1 | |
| **Oxacycloheptadec-7-en-2-one** | |
| 1-Oxa-7-cycloheptadecen-2-one; 16-Hydroxy-6-hexadecenoic acid lactone; 16-Hydroxy-6-hexadecenoic acid ω-lactone | |

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).

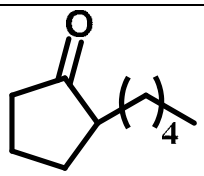
| ***AMYL CINNAMYL ALCOHOL*** |  |
|---|---|
| CAS # 101-85-9 | |
| EC # 202-982-8 | |
| **2-(Phenylmethylene)-heptan-1-ol,** | |
| 2-Benzylidene- (6CI,8CI)1-heptanol; 2-Amyl-3-phenyl-2-propen-1-ol; 2-Benzylidene-1-heptanol; 2-Pentyl-3-phenyl-2-propen-1-ol; Buxinol; α-Amylcinnamic alcohol; α-Amylcinnamyl 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).

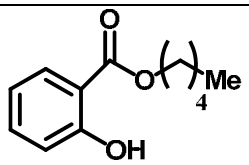
Additional information: A RIFM review is available (35) where selected clinical studies published until 1994 were considered.

| ***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: /

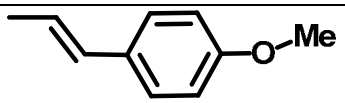
| ***AMYL SALICYLATE*** |  |
|---|---|
| CAS # 2050-08-0 | |
| EC # 218-080-2 | |
| **Pentyl-2-hydroxybenzoate** | |
| Amyl ester salicylic acid, (4CI); Pentyl ester salicylic acid, (6CI,8CI); 2-Hydroxybenzoic acid pentyl ester; Amyl salicylate; NSC 403668; NSC 44877; NSC 46125; Pentyl salicylate | |

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)

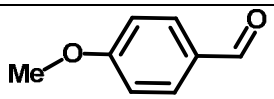
| ***trans-ANETHOLE*** |  |
|---|---|
| CAS # 4180-23-8 | |
| EC # 224-052-0 / 203-205-5 | |
| **1-Methoxy-4-(1E)-1-propen-1-yl-benzene** | |
| (E)-p-Propenyl-anisole (8CI); (E)-1-Methoxy-4-(1-propenyl)-benzene; 1-Methoxy-4-(1E)-1-propenyl-benzene (9CI); (E)-1-(4-Methoxyphenyl)propene; (E)-1-p-Methoxyphenylpropene; (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

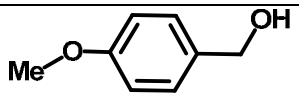
and fennel oils (38)

| ***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).

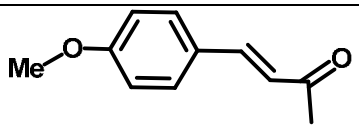
| ***ANISYL ALCOHOL*** |  |
|---|---|
| CAS # 105-13-5 | |
| EC # 203-273-6 | |
| **4-Methoxy-benzenemethanol** | |
| p-Methoxy-benzyl alcohol (8CI); (4-Methoxyphenyl)methyl alcohol; 4-(Hydroxymethyl)anisole; 4-(Methoxyphenyl)methanol; 4-Methoxy-α-hydroxytoluene; 4-Methoxybenzenemethanol; 4-Methoxybenzyl 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).

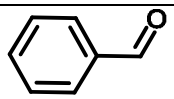
Additional information: /

| ***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)

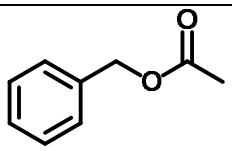
Additional information: /

| ***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: /

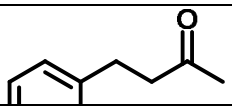
| ***BENZYL ACETATE*** |  |
|---|---|
| CAS #140-11-4 | |
| EC # 205-399-7 / 202-940-9 | |
| **Benzyl actetate** | |
| Benzyl ester acetic acid; Benzyl alcohol, acetate (6CI); (Acetoxymethyl)benzene; Benzyl ethanoate; NSC 4550; Phenylmethyl acetate; Methyl Phenylacetate; α-Acetoxytoluene ; Methyl alpha-Toluate | |

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).

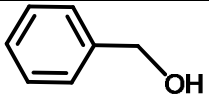
| ***BENZYL ACETONE*** |  |
|---|---|
| CAS # 2550-26-7 | |

| | |
|---|---|
| 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).

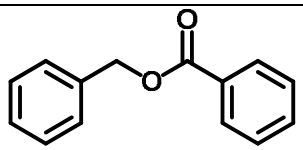
Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.3% (95% CI: 0.1 – 0.7%) positive reactions in 2166 consecutively PTed patients (4). In the Groningen 2009 study, n=1, i.e. 0.3% (95% CI: 0.01 – 1.7%) had positive reactions to this allergen (6).

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:

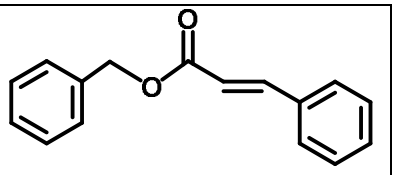
In the "background information" section of the 1999 opinion, benzyl benzoate is classified as "less frequently reported allergen"; in several studies, only single cases had been reported in each, except for patients sensitive to MP (33).

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).

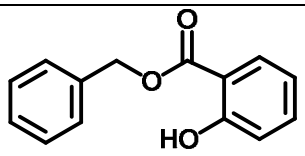
| ***BENZYL CINNAMATE*** |  |
|---|---|
| CAS # 103-41-3 | |
| EC # 203-109-3 | |
| **Benzyl 3-phenylprop-2-enoate** | |
| Benzyl ester cinnamic acid; 3-phenyl-phenylmethyl ester 2-propenoic acid; 3-Phenyl-2-propenoic acid benzyl ester; Benzyl 3-phenylpropenoate; Benzyl γ-phenylacrylate; Cinnamein; NSC 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

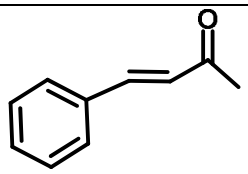
Clinical data:

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 | |

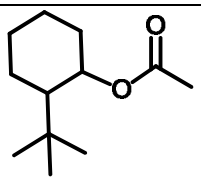
Current regulation: Annex II, n° 356

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: /

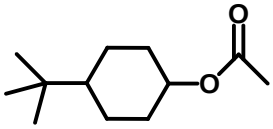
| ***2-TERT-BUTYLCYCLOHEXYL ACETATE*** |  |
|---|---|
| CAS # 88-41-5 | |
| EC # 201-828-7 | |
| **2-(1,1-dimethylethyl)cyclohexyl acetate** | |
| Cyclohexanol, 2-(1,1-dimethylethyl)-, acetate ; Cyclohexanol, 2-Tert-butyl-, acetate; 2-Tert-Butylcyclohexanol acetate; Verdox; o-Tert-Butylcyclohexyl acetate | |

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).

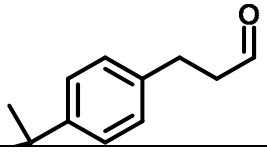
| ***4-TERT-BUTYLCYCLOHEXYL ACETATE*** |  |
|---|---|
| CAS # 32210-23-4 | |
| EC # 250-954-9 | |
| **4-(1,1-Dimethylethyl)cyclohexyl acetate** | |
| Boisinol A 464D; Cyclohexanol, 4-tert-Butyl-, acetate; Cyclohexanol, 4-(1,1-Dimethylethyl)-, acetate; 4-(1,1-Dimethylethyl)cyclohexyl acetate; 4-tert-Butylcyclohexanol 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 | |

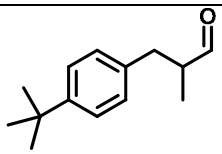
| **4-(1,1-Dimethylethyl)-benzenepropanal** | |
|---|---|
| p-tert-Butyl-hydrocinnamaldehyde; 3-(4-tert-Butylphenyl)propanal; Bourgeonal; p-tert-Butyldihydrocinnamaldehyde | |

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:

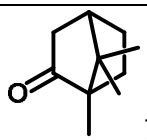
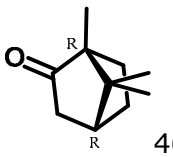
In the "background information" section of the 1999 opinion, lilial is classified as "less frequently reported allergen"; with 2 cases of contact allergy reported in 1 study of 176 eczema patients and 1 case with contact allergy to Lilial from a deodorant; a number of other reported positive cases were 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:

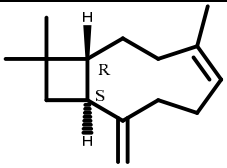
It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

| ***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)<br>**(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<br>**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 | |

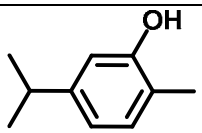
Current regulation: /

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 | |

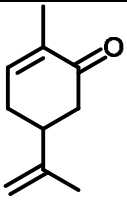
Current regulation: /

Clinical data:

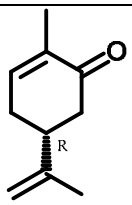
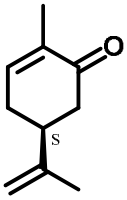
The DeGroot 1985 study identified 2 (1.1%) positive reactions among 179 patients using a 5% PT preparation of this compound – these reactions may have been at least partly due to an "excited back syndrome" and are thus of limited evidence (25). Meynadier et al. [11] patch tested 28 patients with contact 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)<br>**(5S)-2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-one** (2244-16-8) |  <br>6485-40-1<br>  <br>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<br>**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<br>**2244-16-8**: (S)-(+)-p-Mentha-6,8-dien-2-one; (+)-Carvone; (S)-(+)-Carvone; (S)-(+)-p-Mentha-6,8-dien-2-one; (S)-Carvone; (+)-Carvone; D-(+)-Carvone; D-Carvone; Talent; d-1-Methyl-4-isopropenyl-6-cyclohexen-2-one; (S)-2-Methyl-5-(1-methylvinyl)cyclohex-2-en-1-one; d-Carvone | |

Current regulation: /

Clinical data:

Cases of allergic contact cheilitis due to L-carvone in toothpastes have been reported (67-69). In an earlier study, 15 of 541 (2.8%) of consecutive PT patients tested also with L-Carvone (5% pet.) exhibited positive reactions, which were (i) associated with positive PT results to *Compositae* mix and (ii) mostly were not considered clinically relevant. Upon re-testing with lower concentrations (2% and 1% pet.) only 2 of 8 patients thus tested were positive (70).

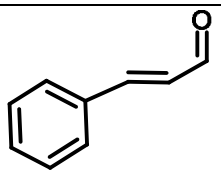
"Carvone has occasionally been reported as an allergen, usually in flavourings. Isomers of carvone have been either a mint or a rye flavour and aroma. We report a woman with positive patch-test reactions to carvone (newly added to the North American Contact Dermatitis Group standard series) and dermatitis on the head. She had used a hair conditioner with a "mint" scent, and the dermatitis resolved when she discontinued using this product. While the manufacturer would not confirm carvone as an ingredient, the clinical course, patch-test results, and ingredient list strongly suggest that this was a relevant allergen in this case of allergic contact dermatitis"[12]

Additional information:

D-Carvone occurs in caraway seed oil and dill oil in a concentration of up to 60%. L-Carvone is a component of the oil from Mentha spicata (spearmint).

R-Carvone is identified as a secondary oxidation product in autoxidized limonene (71). However, it is not a major allergen in this oxidation mixture and only one of 30 patients with known contact allergy to oxidized R- limonene reacted when tested with carvone (3% pet.) (72). Experimental findings in guinea pigs show no cross reactivity between R- and S carvone, but both enantiomers were found to be equally strong sensitizers (73).

[12] http://www.ncbi.nlm.nih.gov/pubmed/20233552

| ***CINNAMAL*** |  |
|---|---|
| CAS # 104-55-2 | |
| EC # 203-213-9 | |
| **3-Phenyl-2-propenal** | |
| Cinnamaldehyde; 3-Phenyl-2-propen-1-al; 3-Phenyl-2-propenaldehyde; 3-Phenylacrolein; 3-Phenylacrylaldehyde; 3-Phenylpropenal; Abion CA; Benzylideneacetaldehyde; Cassia aldehyde; Cinnacure; Cinnamal; Cinnamic aldehyde; Cinnamite; Cinnamyl aldehyde; NSC 16935; NSC 40346; Phenylacrolein; Zimtaldehyde; β-Phenylacrolein | |

Current regulation: Annex III, part 1, n° 76

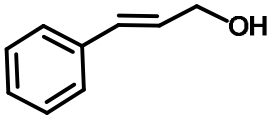
Clinical data:
In the "background information" section of the previous opinion (33), cinnamal, one of the 8 constituents of the FM I, is classified as frequent allergen, causing allergic reactions in a notable persons with eczema from cosmetic products in several studies (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 1.0% (95% CI: 0.6 – 1.6%) positive reactions in 2063 consecutively PTed patients (4). In the Groningen 2009 study, 1.6% (95% CI: 0.5 – 3.6%) had positive reactions to cinnamal (6). In a study by the North American Contact Dermatitis Group, no significant trend of cinnamal contact sensitisation in the consecutive patients analysed was observed between 1984 (5.9% pos.) and 2000 (3.6% pos.); tested at 1% pet. (74). In the An 2005 study, 7 of 422 consecutive patients, i.e., 1.7%, had positive reaction (13). The Belsito 2006 study (20) yielded 1.7% positive reactions. In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 1.9% positive reactions (22). The NACDG study found 3.1% positive reactions in 4435 patients tested (21). The IVDK 2010 study, 1.43% (95% CI: 0.67 – 2.18%) of 1214 consecutively tested patients reacted to the compound, while 2.64% (95% CI: 2.16 – 3.13%) of 4527 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=7 reacted positively to cinnamal (48).

While, in addition to typical ACD due to contact sensitisation, immediate reactions to some fragrance compounds (and MPR, see below) are observed not infrequently, such immediate type reactions may rarely be very severe (anaphylaxis) and possibly immunologically mediated, as illustrated by the case of a 42 year old nurse with anaphylaxis (maximum grade of contact urticaria syndrome) 20 min after application of cinnamal (75). Following industrial use as "odour masking" agent, cinnamal caused occupational ACD in an exposed worker (76).

Additional information:

A specific RIFM review is available (77); another RIFM review addresses several cinnamic compounds (78).

| ***CINNAMYL ALCOHOL*** |  |
|---|---|
| CAS # 104-54-1 | |
| EC # 203-212-3 | |
| **3-Phenyl-2-propen-1-ol** | |

| | |
|---|---|
| Cinnamyl alcohol; 1-Phenyl-3-hydroxy-1-propene; 1-Phenylprop-1-en-3-ol; 3-Hydroxy-1-phenylprop-1-ene; 3-Phenyl-2-propenol; 3-Phenylallyl alcohol; Cinnamic alcohol; NSC 623440; NSC 8775; Styrone; Styryl alcohol; Styryl carbinol; γ-Phenylallyl alcohol | |

Current regulation: Annex III, part 1, n° 69

Clinical data:

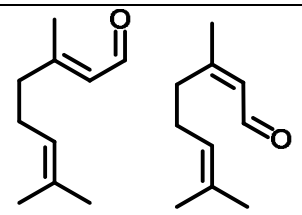
In the "background information" section of the previous opinion (33), cinnamyl alcohol, one of the 8 constituents of the FM I, is classified as frequent allergen, causing allergic reactions in a notable persons with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.6% (95% CI: 0.3 – 1.1%) positive reactions in 2063 consecutively PTed patients (4). In the Groningen 2009 study, 2.5% (95% CI: 1.1 – 4.9%) had positive reactions to cinnamyl alcohol, tested at 2% pet., i.e., twice the commonly used concentration (6). As test concentrations of up to 5% are apparently non-irritating (de Groot et al. after (33)), the latter data can be regarded as valid. In the An 2005 study, 13 of 422 consecutive patients, i.e., 3.1%, had positive reaction (13) (test concentration 2%). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 1.5% positive reactions (22). The IVDK 2010 study, 0.73% (95% CI: 0.17 – 1.30%) of 1214 consecutively tested patients reacted to the compound, while 2.36% (95% CI: 1.89 – 2.83%) of 4502 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=12 reacted positively to cinnamyl alcohol (48).

Additional information:

In a recent experimental study protein-cinnamal adducts were detected in skin homogenates treated with cinnamal and cinnamyl alcohol but not with alpha-amyl cinnamal. This suggests that there is a common hapten involved in cinnamal and cinnamyl alcohol sensitization, in line with the observation of a marked concordance upon patch testing (7, 79), and that metabolic activation (to cinnamal) is involved in the latter. Conversely, there does not appear to be a common hapten for cinnamal and alpha-amyl cinnamal (80), again in line with the observations in the IVDK 2010 study (7).

A RIFM review is available (81)

| | |
|---|---|
| ***CITRAL*** |  |
| CAS # 5392-40-5 | |
| EC # 226-394-6 | |
| **3,7-Dimethyl-2,6-octadienal** | |
| 3,7-Dimethyl-2,6-octadien-1-al; Citral; Citral PQ Extra; Lemarome N; Lemsyn GB; NSC 6170 | **Citral** = isomeric mixture of Geranial and Neral |

Current regulation: Annex III, part 1, n° 70

Clinical data:

In the "background information" section of the previous opinion (33), citral is classified as frequent allergen, causing about 1% allergic reactions in consecutive PT patients, and being a proven cause of contact allergic reactions in 2.6% patients with eczema from

cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the Frosch 2002 a study yielded 1.1% positive (and 1.3% doubtful) reactions among the 1855 consecutive patients tested (16). In a study on 586 consecutive patients with hand eczema it has been noted that citral (2% pet.) not only caused (mostly weak) positive PT reactions, but far more often irritant reactions (n=82 vs. n=28). It was hypothesised that this very property could contribute to citral's sensitising potential (82). In the EU 2005 study, 12 of 1701 patients (0.7%, 95% CI: 0.4 – 1.2%) reacted positively to 2% citral in pet. (10). The IVDK 2007 study yielded 0.6% (95% CI: 0.3 – 1.1%) positive reactions in 2021 consecutively PTed patients; 10 of 13 citral positive patients also reacted positively to geraniol (4). In the Groningen 2009 study, 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen (6). In the deGroot 2000 multicentre study, 19 of 1825 consecutive patients tested positively to citral (2% pet.), 4 of whom did not react positively to the FM I (12). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had positive reaction (13) (test concentration 2%). In the Malten 1984 study, neral at 1% in pet. yielded 2.6% positive reactions in 182 patients (24). In a study from Alicante, Spain, 86 selected patients were tested with citral, yielding 2 positive reactions (48).

Citral in a lip salve has been reported to have caused longstanding, recurrent allergic contact cheilitis in a 30 year old female patient, diagnosed by a strong positive reaction to the FM II, followed by a strong positive reaction to citral (83).

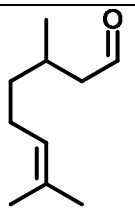
Additional information:

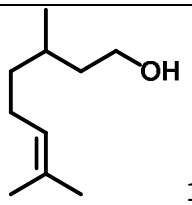
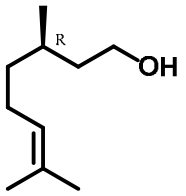
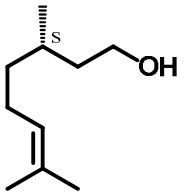
Citral is the mixture of two isomers: cis-citral (neral) and trans-citral (geranial).

Geranial forms oxidation product with increased sensitizing capacity both via spontaneous autoxidization at air exposure and via metabolic oxidation (Hagvall L. Thesis 2009: http://hdl.handle.net/2077/18951).

Geranial and neral have been identified as secondary oxidation products when geraniol autoxidizes (84). They have also been identified as metabolites of geraniol (85). This explains the simultaneous reactions to geraniol and citral seen by (4).

It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

| ***CITRONELLAL*** |  |
|---|---|
| CAS # 106-23-0 | |
| EC # 203-376-6 | |
| **3,7-Dimethyl-6-octenal** | |
| (±)-Citronellal; 2,3-Dihydrocitral; 3,7-Dimethyloct-6-en-1-al; Citronellal; NSC 46106; Rhodinal; dl-Citronellal; β-Citronellal | |
| Current regulation: / | |
| Clinical data: / | |
| Additional information: A compound of essential oils of citrus fruits, namely grapefruit, but also contained in "citronella oil" and oil of Melissa. | |

| ***CITRONELLOL*** |  106-22-9  1117-61-9  7540-51-4 |
|---|---|
| CAS # 106-22-9 / 1117-61-9 / 7540-51-4 | |
| EC # 247-737-6 / 214-250-5 / 231-415-7 | |
| **3,7-Dimethyl-6-octen-1-ol** (106-22-9); **(3R)-3,7-Dimethyl-6-octen-1-ol** (1117-61-9); **(3S)-3,7-Dimethyl-6-octen-1-ol** (7540-51-4) | |
| **106-22-9**: (±)-3,7-Dimethyl-6-octen-1-ol; (±)-Citronellol; (±)-β-Citronellol; 2,3-Dihydrogeraniol; 2,6-Dimethyl-2-octen-8-ol; Cephrol; Citronellol; Citronellol 950; DL-Citronellol; Dihydrogeraniol; NSC 8779; Rodinol; dl-Citronellol; β-Citronellol<br>**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<br>**7540-51-4**: (-)-3,7-Dimethyl-6-octen-1-ol; (-)-(S)-Citronellol; (-)-Citronellol; (-)-β-Citronellol; (S)-(-)-Citronellol; (S)-(-)-β-Citronellol; (S)-3,7-Dimethyl-6-octen-1-ol; (S)-Citronellol; (S)-β-Citronellol; L-Citronellol; l-Citronellol | |
| Current regulation: Annex III, part 1, n° 86 | |

Clinical data:

In the "background information" section of the 1999 opinion, citronellol is classified as "less frequently reported allergen"; with few cases of contact allergy reported in the literature (33).

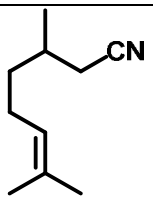
Since the last SCCNFP-opinion of 1999, in the Larsen 2002 c study, „DL citronellol" (5% in pet.) elicited positive PT reactions in 8.7% of the patients (1). In 1855 consecutive patients of the Frosch 2002 a study, 0.4% positive reactions were noted (16). In the EU 2005 study, 4 of 1701 patients (0.2%, 95% CI: 0.06 – 0.6%) reacted positively to 1%

citronellol in pet.; at the same concentration, n=23 doubtful or irritant reactions were observed (10). The IVDK 2007 study yielded 0.5% (95% CI: 0.2 – 0.9%) positive reactions in 2003 patients consecutively PTed (4). In the Groningen 2009 study, n=1, i.e. 0.3% (95% CI: 0.01 – 1.7%) had positive reactions to this allergen, tested at only 2% pet. (6). The Larsen 2001 study yielded 5.6% positive reactions to l-citronellol (5% pet.) in 178 patients with known contact allergy to fragrance ingredients (19).

Additional information:

Citronellol autoxidizes spontaneously in contact with air in the same way as linalool forming allergenic primary oxidation products, hydroperoxides (AT Karlberg, personal communication, 2011).

RIFM reviews have been published regarding L-citronellol (86), D-citronellol (87) and DL-citronellol (88). Another review is available by Hostynek and Maibach (89). It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

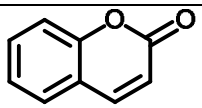
| ***CITRONELLYL NITRILE*** |  |
|---|---|
| CAS # 51566-62-2 | |
| EC # 257-288-8 | |
| **3,7-Dimethyl-6-octenenitrile** | |
| 3,7-Dimethyl-6-octenonitrile (REACH, EINECS, INCI); Agrunitril; Agrunitrile; Citronellyl nitrile | |



Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010)

| ***COUMARIN*** |  |
|---|---|
| CAS # 91-64-5 | |
| EC # 202-086-7 | |
| **2H-1-Benzopyran-2-one** | |
| 1,2-Benzopyrone; 2-Chromenone; 2-Propenoic acid, 3-(2-hydroxyphenyl)-, δ-lactone;5,6-Benzo-2-pyrone; Benzo-α-pyrone; Coumarinic anhydride; NSC 8774; Rattex; Tonka bean camphor; cis-o-Coumarinic acid lactone; o-Hydroxycinnamic acid lactone | |

Current regulation: Annex III, part1, n° 77

Clinical data:

In the "background information" section of the previous opinion (33), coumarin is classified as frequent allergen, causing allergic reactions in about 0.4 – 0.8% in consecutive PT patients, and causing contact allergic reactions in 0.8-10% of patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, in the Frosch 2002 a study, 0.3% positive PT reactions to consecutive patients were noted (16). In the EU 2005 study, none of the

1701 patients reacted positively to 5% coumarin in pet., while 7 doubtful or irritant reactions were observed (10). The IVDK 2007 study yielded 0.4% (95% CI: 0.2 – 0.8%) positive reactions in 2020 consecutively PTed patients (4). In the Groningen 2009 study, 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen (6). In the deGroot 2000 study, 13 of 1825 consecutive patients reacted positively to coumarin (5% pet.) (12).

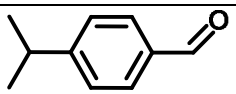
V. Mutterer et al. present the case of a 44 year old patient in whom coumarin was identified as culprit allergen by controlled ROAT testing with 1%, after having caused dermatitis by the use of a deodorant containing coumarin at 0.23% and an EdT (90).

Additional information:

Coumarin is found in several plant families, including *Melilotus* and *Galium*, e.g., *Galium odoratum* (sweet woodruff), however, also in oil of lavender, lovage and others (53).

Researchers from INSERM and "Rhodia Organique, Lyon , France" observed that pure coumarin is not an allergen in the LLNA, however, commercially available materials, containing "contaminants" (3,4-dihydrocoumarin, 6-chlorocoumarin and 6,12-epoxy-6H,12H-dibenzo[b,f][1,5] dioxocin, were identified as weak and moderate sensitisers, resp. (91).

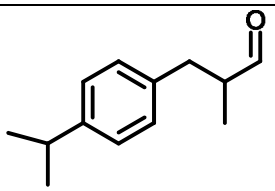
Coumarin is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

| ***CUMINALDEHYDE*** |  |
|---|---|
| CAS # 122-03-2 | |
| EC # 204-516-9 | |
| **4-(1-Methylethyl)-benzaldehyde** | |
| 4-Isopropylbenzaldehyde; p-Isopropylbenzaldehyde; 4-(Propan-2-yl)benzaldehyde; 4-Isopropylphenylcarboxaldehyde; Cumaldehyde; Cumic aldehyde; Cuminal; Cuminaldehyde; Cuminic aldehyde; Cuminyl aldehyde; NSC 4886; p-Cumic aldehyde; p-Isopropylbenzaldehyde; p-Isopropylbenzenecarboxaldehyde | |

Current regulation: /

Clinical data:
The DeGroot 1985 study identified 3 (1.7%) positive reactions among 179 patients using a 15% PT preparation of cuminaldehyde (25).

Additional information: …

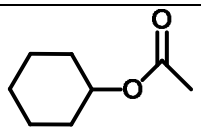
| ***CYCLAMEN ALDEHYDE*** |  |
|---|---|
| CAS # 103-95-7 | |
| EC # 203-161-7 | |
| **α-Methyl-4-(1-methylethyl)-benzenepropanal** | |
| p-Isopropyl-α-methyl-hydrocinnamaldehyde; 2-Methyl-3-(4-isopropylphenyl)propionaldehyde; 2-Methyl-3-(p-isopropylphenyl)propionaldehyde; 3-(4-Isopropylphenyl)-2-methylpropanal; 3-(p-Isopropylphenyl)-2- | |

| | |
|---|---|
| methylpropionaldehyde; 3-p-Cumenyl-2-methylpropionaldehyde(REACH, EINECS); 4-Isopropyl-α-methylhydrocinnamic aldehyde; Cyclamal; Cyclamen aldehyde; Cyclosal; Cyclosal perfume; Cymal; p-Isopropyl-α-methylhydrocinnamaldehyde; α-Methyl-4-(1-methylethyl)benzenepropanal; α-Methyl-p-isopropylhydrocinnamaldehyde | |

Current regulation: …

Clinical data:
/

Additional information: It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

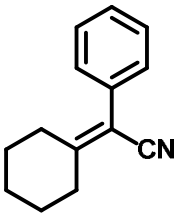
| ***CYCLOHEXYL ACETATE*** |  |
|---|---|
| CAS # 622-45-7 | |
| EC # 210-736-6 | |
| **Cyclohexyletanoat** | |
| Acetic acid cyclohexanyl ester; Acetoxycyclohexane; Cyclohexyl acetate; NSC 8772 | |

Current regulation: /

Clinical data:

In the Larsen 2002 c study, 0.5% positive reactions among 218 patients with know contact allergy to fragrance ingredients were observed (1).

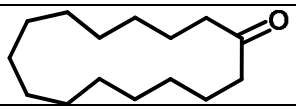
Additional information: A RIFM review is available (92).

| ***alpha-CYCLOHEXYLIDENE BENZENEACETONITRILE*** |  |
|---|---|
| CAS # 10461-98-0 | |
| EC # 423-740-1 | |
| **α-Cyclohexylidenebenzeneacetonitrile** | |
| alpha-Cyclohexylidene-benzeneacetonitrile (REACH); Δ1α-Phenyl-α-Cyclohexaneacetonitrile; 2-Cyclohexylidene-2-phenylacetonitrile; NSC 408284; Peonile (REACH) | |

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

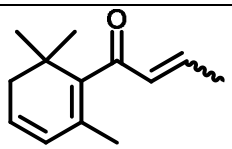
| ***CYCLOPENTADECANONE*** |  |
|---|---|
| CAS # 502-72-7 | |

| | |
|---|---|
| EC # 207-951-2 | |
| **Cyclopentadecanone** | |
| CPE 218; Exaltone; NSC 63900; Normuscon; Normuscone | |

Current regulation: /

Clinical data:
In the Larsen 2001 study, n=3, i.e., 1.7% positive reactions were observed to the compound, tested 5% pet., in 178 pateints with known contact allergy to fragrance ingredients (19).

Additional information: …

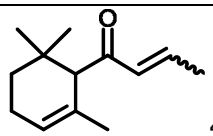
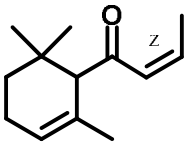
| | |
|---|---|
| ***DAMASCENONE***<br>***ROSE KETONE-4*** (Not officially an INCI Name but Perfuming Name; Damascenone as such is not listed in CosIng) |  |
| CAS # 23696-85-7 | |
| EC # 245-833-2 | |
| **1-(2,6,6-Trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one** | |
| 1-(2,6,6-Trimethyl-1,3-cyclohexadienyl)-2-buten-1-one; 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<br>**23726-94-5**: (Z)-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one; (Z)-α-Damascone; cis-α-Damascone | |

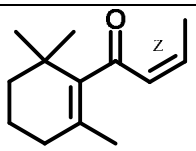
Current regulation: Annex III, part1, n° 157 (max. conc. 0.02%)

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.

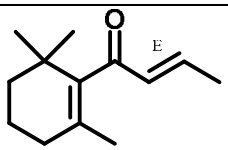
| ***cis-beta-DAMASCONE*** |  |
|---|---|
| CAS # 23726-92-3 | |
| EC # 245-843-7 | |
| **(2Z)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one** | |
| (Z)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one; (Z)-β-Damascone | |

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.

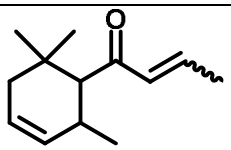
| ***trans-beta-DAMASCONE*** |  |
|---|---|
| CAS # 23726-91-2 | |
| EC # 245-842-1 | |
| **(2E)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one** | |
| (E)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one; (E)-β-Damascone; Damascone beta; trans-2,6,6-Trimethyl-1-crotonylcyclohex-1-ene; trans-β-Damascone; 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%).

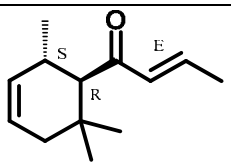
| ***delta-DAMASCONE*** |  |
|---|---|
| CAS # 57378-68-4 | |
| EC # 260-709-8 | |
| **1-(2,6,6-Trimethyl-3-cyclohexen-1-yl)-2-buten-1-one** | |
| δ-Damascone | |

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.

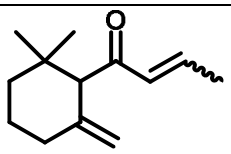
| ***trans-trans-delta-DAMASCONE*** |  |
|---|---|
| CAS # 71048-82-3 | |
| EC # 275-156-8 | |
| **(2E)-rel-1-[(1R,2S)-2,6,6-Trimethyl-3-cyclohexen-1-yl]- 2-buten-1-one** | |
| [1α(E),2β]-1-(2,6,6-Trimethyl-3-cyclohexen-1-yl)-2-buten-1-one; trans-δ-Damascone; δ-Damascone; trans, trans-δ-Damascone | |

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%).

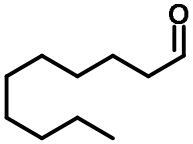
| ***gamma-DAMASCONE*** |  |
|---|---|
| CAS # 35087-49-1 | |
| EC # 481-910-9 | |
| **1-(2,2-Dimethyl-6-methylenecyclohexyl)-2-buten-1-one** | |
| γ-Damascone | |

Current regulation: /

Clinical data: /

Additional information:

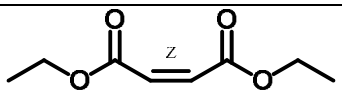
A RIFM review is available (102), citing 1 positive Buehler test and 1 LLNA study yielding an EC3 value of 4.6%

| ***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).

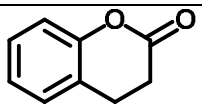
| ***DIETHYL MALEATE*** |  |
|---|---|
| CAS # 141-05-9 | |
| EC # 205-451-9 | |
| (**2Z)-Diethyl but-2-enedioate** | |
| 2-Butenedioic acid (2Z)-, diethyl ester; 2-Butenedioic acid (Z)-, diethyl ester; Maleic acid, diethyl ester; (2Z)-2-Butenedioic acid diethyl ester; Diethyl (Z)-2-butenedioate; Ethyl 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: /

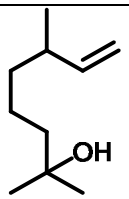
| ***DIHYDROCOUMARIN*** |  |
|---|---|
| CAS # 119-84-6 | |
| EC # 204-354-9 | |
| **3,4-Dihydro-2H-1-benzopyran-2-one** | |
| Hydrocoumarin; Hydrocinnamic acid, o-hydroxy-, δ-lactone; 2-Chromanone; 3,4-Dihydro-1H-benzopyran-2-one; 3,4-Dihydrocoumarin; Dihydrocoumarin; Melilotin; Melilotin (coumarin); 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) | |

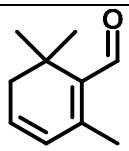
Current regulation: /

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).

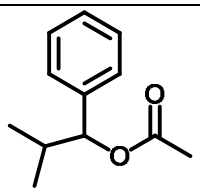
| ***2,3-DIHYDRO-2,2,6-TRIMETHYLBENZALDEHYDE*** |  |
|---|---|
| CAS # 116-26-7 | |
| EC # 204-133-7 | |
| **2,6,6-Trimethyl-1,3-cyclohexadiene-1-carboxaldehyde** | |
| 2,2,6-Trimethyl-4,6-cyclohexadien-1-aldehyde; 2,6,6-Trimethyl-1,3-cyclohexadiene-1-aldehyde; Safranal | |

Current regulation: /

Clinical data: /

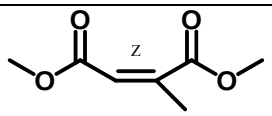
Additional information:

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:<br>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).<br><br>Additional information: It is a "top 100" substance (IFRA, pers. comm.2010). |

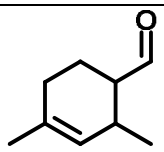
| ***DIMETHYL CITRACONATE*** |  |
|---|---|
| CAS # 617-54-9 | |
| EC # | |
| **(2Z)-Diethyl-2-metyl-but-2-enedioate** | |
| (2Z)-2-methyl-2-Butenedioic acid, dimethyl ester; 2-Butenedioic acid, 2-methyl-, dimethyl ester, (Z)-; Citraconic acid, dimethyl ester; Dimethyl methylmaleate; Methylmaleic acid, 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: …

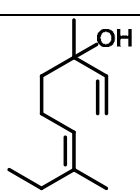
| ***2,4-DIMETHYL-3-CYCLOHEXEN-1-CARBOXALDEHYDE*** |  |
|---|---|
| CAS # 68039-49-6 | |
| EC # 268-264-1 | |
| **2,4-Dimethyl-cyclohex-3-ene-1-carboxaldehyde** | |
| (Z)-Vertocitral C; 2,4-Dimethyl-3-cyclohexene-1-carboxaldehyde; 2,4-Dimethyl-3-cyclohexenecarboxaldehyde; 2,4-Dimethyl-3-cyclohexenylcarbaldehyde; 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).

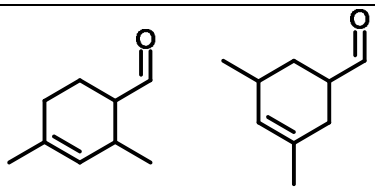
| ***3,7-DIMETHYL-1,6-NONADIEN-3-OL*** |  |
|---|---|
| CAS # 10339-55-6 | |
| EC # 233-732-6 | |
| **(7Z)-3,7-Dimethyl-1,6-nonadien-3-ol** | |
| Ethyl linalool; Methyllinalool | |

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).

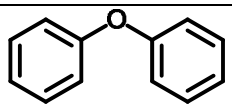
| ***DIMETHYLTETRAHYDRO BENZALDEHYDE*** |  |
|---|---|
| CAS # 68737-61-1 | |
| EC # 272-113-5 | |
| **2,4-Dimethyl-cyclohex-3-ene-1-carboxaldehyde**<br>**3,5-Dimethyl-cyclohex-3-ene-1-carboxaldehyde** | 2,4- 3,5- |
| Hivertal; Vertocitral | |

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).

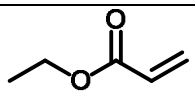
Additional information: /

| ***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 | |

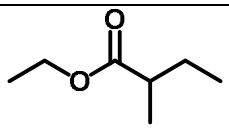
Current regulation: Annex II, n° 435

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).

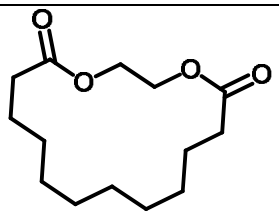
Additional information: /

| ***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).

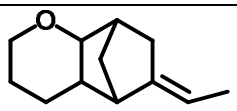
| ***ETHYLENE DODECANEDIOATE*** |  |
|---|---|
| CAS # 54982-83-1 | |
| EC # 259-423-6 | |
| **1,4-Dioxacyclohexadecane-5,16-dione** | |
| Cyclic ethylene dodecanedioate; Ethylene dodecanedioate; Musk 144; Musk C-14 | |

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).

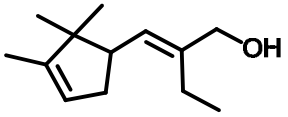
Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

| ***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).

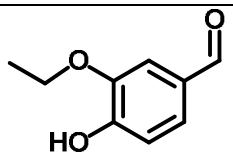
Additional information: /

| ***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;<br>Bacdanol; Bangalol; Dartanol; Finanol; Levosandol; Radjanol; Sanjinol | |

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

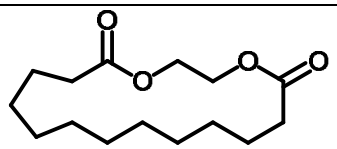
| ***ETHYL VANILLIN*** |  |
|---|---|
| CAS # 121-32-4 | |
| EC # 204-464-7 | |
| **3-Ethoxy-4-hydroxybenzaldehyde** | |
| 2-Ethoxy-4-formylphenol; 3-Ethoxy-4-hydroxybenzaldehyde; 3-Ethylvanillin; 4-Hydroxy-3-ethoxybenzaldehyde; Arovanillon; Bourbonal; Ethavan; Ethovan; Ethylprotal; Ethylvanillin; NSC 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).

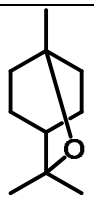
Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

| ***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 | |

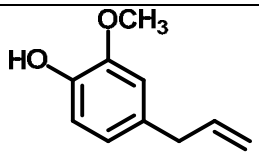
Current regulation: /

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).

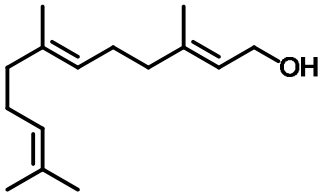
Moreover, eugenol is capable of inducing immediate type reactions of the airways, as illustrated by the well-documented case of a 30 year old hairdresser who developed severe occupational bronchial asthma due to eugenol (107). A case of urticaria after dental treatment with eugenol-containing material 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:

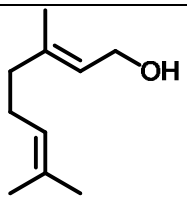
In the "background information" section of the 1999 opinion, farnesol is classified as "less frequently reported allergen"; in 1 study of patients with cosmetic dermatitis 2 cases with contact allergy to farnesol had been reported; in other studies, positive reactions were seen in patients with positive 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).

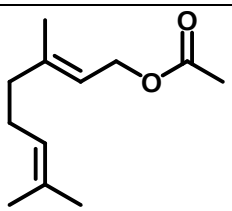
The fact that geraniol also occurs in food flavourings, and can elicit signs and symptoms of manifest contact sensitisation, is illustrated by the case of a 19 year old Japanese woman with cheilitis due to geraniol, improving after avoidance of respective foodstuff (114). A 20 year old Japanese woman 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).

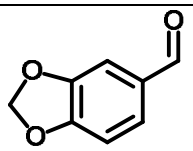
| ***GERANYL ACETATE*** |  |
|---|---|
| CAS # 105-87-3 | |
| EC # 203-341-5 | |
| **(2E)-1-Acetate-3,7-dimethyl-2,6-octadien-1-ol** | |
| (E)-Acetat-3,7-dimethyl-2,6-Octadien-1-ol; Geraniol acetate; (E)-3,7-Dimethyl-2,6-octadien-1-ol acetate; (E)-3,7-Dimethyl-2,6-octadienyl acetate; Acetic acid (2E)-3,7-dimethyl-2,6-octadienyl 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).

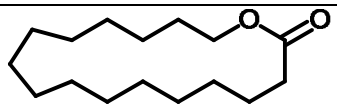
| ***HELIOTROPINE*** |  |
|---|---|
| CAS # 120-57-0 | |
| EC # 204-409-7 | |
| **1,3-Benzodioxole-5-carboxaldehyde** | |
| Piperonal; 2H-Benzo[3,4-d]-1,3-dioxolan-5-ylformaldehyde; 3,4-(Methylenedioxy)benzaldehyde; 3,4-Dihydroxybenzaldehyde methylene ketal; 3,4-Dimethylenedioxybenzaldehyde; 5-Formyl-1,3-benzodioxolane; 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).

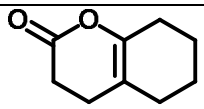
| ***HEXADECANOLACTONE*** |  |
|---|---|
| CAS # 109-29-5 | |
| EC # 203-662-0 | |
| **Oxacycloheptadecan-2-one** | |
| o-Lactone-16-hydroxy-hexadecanoic acid;1,16-Hexadecanolide; 16-Hexadecanolactone; Cyclohexadecanolide; Dihydroambrettolide; Hexadecanoic acid, 16-Hydroxy-, o-lactone; Hexadecanolactone; 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: /

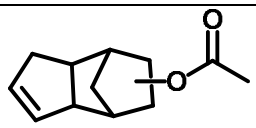
| ***HEXAHYDROCOUMARIN*** |  |
|---|---|
| CAS # 700-82-3 | |
| EC # 211-851-4 | |
| **3,4,5,6,7,8-Hexahydro-2H-1-benzopyran-2-one** | |
| 3,4,5,6,7,8-Hexahydro-coumarin; δ-Lactone-2-hydroxy-1-cyclohexene-1-propanoic acid; 3,4,5,6,7,8-Hexahydrocoumarin; Hexahydrocoumarin; Δ-1,6-2-Oxabicyclo(4.4.0)decen-3-one | |

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 | |

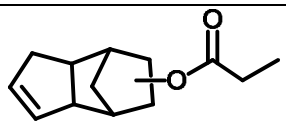
Current regulation: /

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).

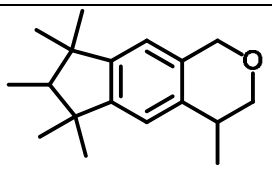
| ***HEXAHYDRO-METHANOINDENYL PROPIONATE*** |  |
|---|---|
| CAS # 68912-13-0 | |
| EC # 272-805-7 | |
| **3a,4,5,6,7,7a-Hexahydro-4,7-methano-1H-indenol propanoate** | |

| | |
|---|---|
| 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: /

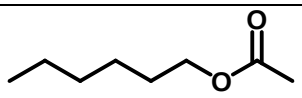
Clinical data:

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).

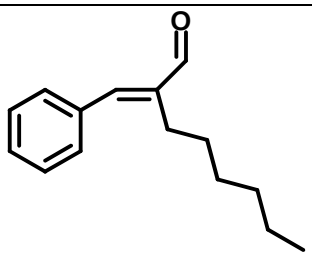
| | |
|---|---|
| ***HEXYL ACETATE*** |  |
| CAS # 142-92-7 | |
| EC # 205-572-7 | |
| **Hexyl ethanoate** | |
| Acetic acid, hexyl ester, Hexyl alcohol, acetate; 1-Hexyl acetate; Exceed 600; Hexyl acetate; Hexyl ester acetic | |

| | |
|---|---|
| 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:

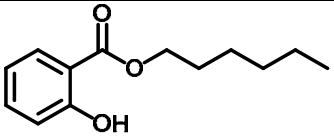
In the "background information" section of the 1999 opinion, hexyl cinnamal (synonymous: alpha-hexyl cinnamal, AHCA) is classified as "less frequently reported allergen"; 2 studies with 1 case and 1 study with 7 cases of contact allergy to this compound in patients with eczema from cosmetic products 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 | |

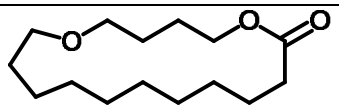
Current regulation: /

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).

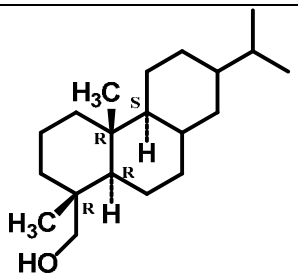
| ***HIBISCOLIDE*** |  |
|---|---|
| CAS # 6707-60-4 | |
| EC # 229-755-6 | |
| **1,6-Dioxacycloheptadecan-7-one** | |
| Undecanoic acid, 11-(4-hydroxybutoxy)-, o-lactone; 12-Oxa-1,16-hexadecanolide; Cervolide; Musk 781; NSC 34741; 12-Oxahexadecan-16-olide | |

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 | |

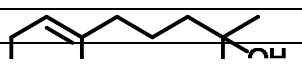
Current regulation: AnnexII, n° 440

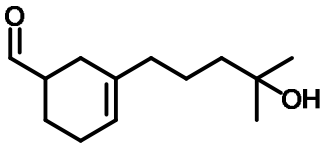
Clinical data:

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)<br>**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:

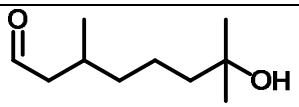
In the "background information" section of the previous opinion (33) HICC is classified as frequent allergen, causing allergic reactions in about 2.8% in consecutive PT patients, two thirds of these being relevant (33).

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

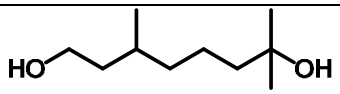
Clinical data:

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 | |

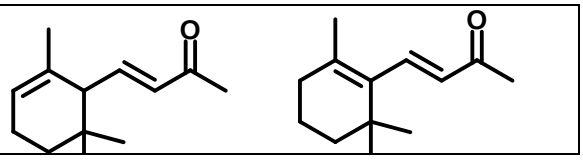
Current regulation: /

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: /

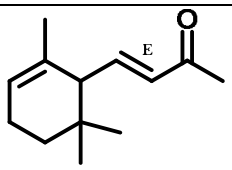
Clinical data: / (see single isomers)

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.

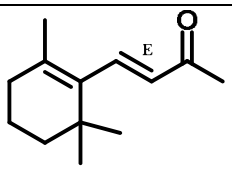
| ***alpha-IONONE*** |  |
|---|---|
| CAS # 127-41-3 | |
| EC # 204-841-6 | |
| **(3E)-4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-Buten-2-one** | |
| (E)-4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-Buten-2-one; (5E)-Ionone; (E)-4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-buten-2-one; (E)-α-Ionone; (±)-trans-α-Ionone; (±)-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: /

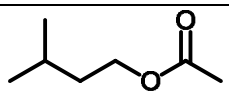
Clinical data:

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).

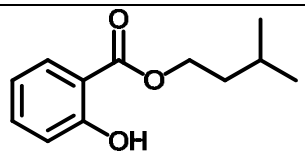
| ***ISOAMYL ACETATE*** |  |
|---|---|
| CAS # 123-92-2 | |
| EC # 204-662-3 | |
| **3-Methylbutyl acetate** | |
| 1-Butanol, 3-methyl-, acetate; Acetic acid, isoamyl ester; Isopentyl alcohol, acetate; 3-Methyl-1-butanol acetate; 3-Methyl-1-butyl acetate; 3-Methylbutyl acetate; 3-Methylbutyl ethanoate; 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 | |

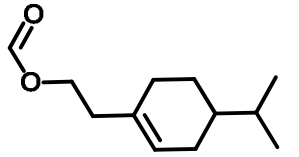
Current regulation: /

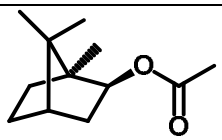
Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

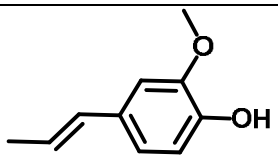
In CosIng, it is listed as "solvent" (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=76810, last accessed 2010-07-13)

| ***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:<br>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).<br>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<br>4-(Isopropyl)cyclohexadiene-1-ethyl methanoate; menthadienyl formate; Menthadiene-7-methyl formate | |
| Current regulation: Annex III, part 1, n° 170 | |
| Clinical data: /<br>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:<br>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).<br>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:

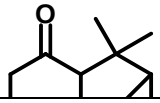
In the "background information" section of the previous opinion (33), isoeugenol, one of the 8 components of the FM I, is classified as frequent allergen, causing allergic reactions in about 1.9% in consecutive PT patients and accounting for 6 to 22% of reactions to the FM I. Allergic reactions had been 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: /

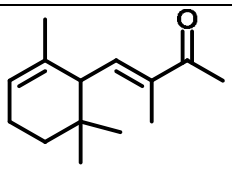
Clinical data:

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).

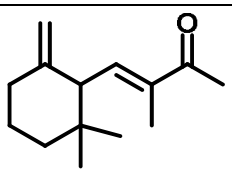
Additional information:

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:

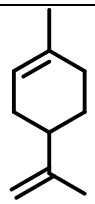
In the "background information" section of the 1999 opinion, "gamma-methylionone" is classified as "less frequently reported allergen"; 1 study with 2 cases and 2 studies with 1 case were found among patients with eczema from cosmetic products (33).

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

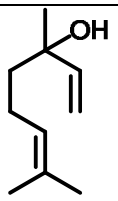
positive patch test reactions to oxidised R-(+)- limonene, e.g.,

- 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<br>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:

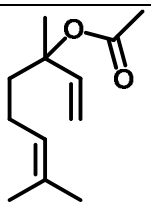
The allergenicity of linalool is closely related to oxidation and the primary oxidation products, the hydroperoxides, are the main allergens (144). In a clinical study 2002-

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).

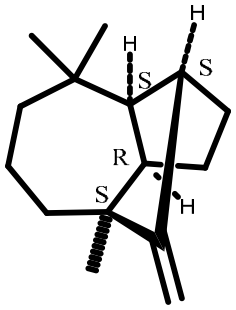
Additional information:

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: /

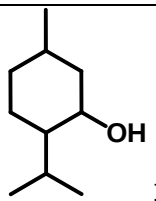
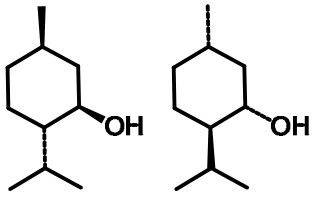
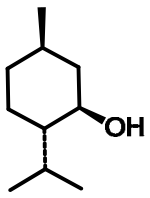
Additional information:

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)<br>**(1R,2S,5R)-rel-5-Methyl-2-(1-methylethyl)-cyclohexanol** (89-78-1)<br>**(1R,2S,5R)-5-Methyl-2-(1-methylethyl)-cyclohexanol** (2216-51-5) | |
| Other names:<br>**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<br>**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).

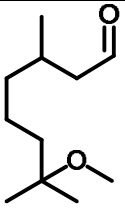
A case of contact allergy to "peppermint and menthol" in a transdermal therapeutic system with flurbiprofen for lumbar pain has been described (155).Moreover, a case of rhinitis caused by different menthol-containing products, diagnostically proven by repeatedly positive urticarial reactions after application 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).

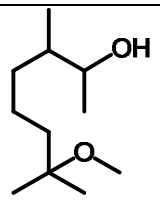
| | |
|---|---|
| ***METHOXYCITRONELLAL*** |  |
| CAS # 3613-30-7 | |
| EC # 222-784-5 | |
| **7-Methoxy-3,7-dimethyl-octanal** | |
| 7-Methoxy-3,7-dimethyloctanal; 7-Methoxy-6,7-dihydrocitronellal; 7-Methoxycitronellal; Methoxycitronellal; Methoxydihydrocitronellal | |

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 | |

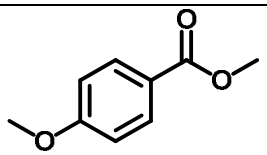
Current regulation: /

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).

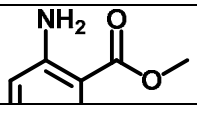
| ***METHYL p-ANISATE*** |  |
|---|---|
| CAS # 121-98-2 | |
| EC # 204-513-2 | |
| **Methyl-4-methoxybenzoate** | |
| p-Anisic acid, methyl ester; 4-(Methoxycarbonyl)anisole; 4-Methoxybenzoic acid methyl ester; Methyl p-anisate; Methyl p-methoxybenzoate; NSC 7324; p-Methoxybenzoic acid methyl ester | |

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 | |

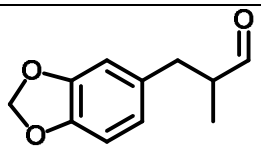
| | |
|---|---|
| EC # 205-132-4 | |
| **Methyl 2-aminobenzoate** | |
| Anthranilic acid, methyl ester; 2-(Methoxycarbonyl)aniline; 2-Aminobenzoic acid methyl ester; 2-Carbomethoxyaniline; Bird Shield; Grain 96-1; Methyl 2-aminobenzoate; Methyl 6-aminobenzoate; Methyl anthranilate; Methyl o-aminobenzoate; 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).

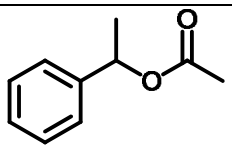
Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

| | |
|---|---|
| ***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).

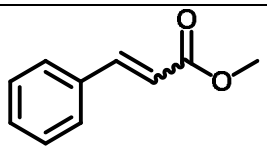
| | |
|---|---|
| ***METHYLBENZYL ACETATE*** |  |
| CAS # 93-92-5 | |
| EC # 202-288-5 | |
| **1-Phenylethyl acetate** | |
| Benzenemethanol, α-methyl-, acetate ; Benzyl alcohol, α-methyl-, acetate ; (±)-Styrallyl acetate; (±)-α-Methylbenzyl acetate; (±)-α-Phenethyl acetate; 1-Acetoxy-1-phenylethane; 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 | |

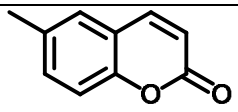
Current regulation: /

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 | |

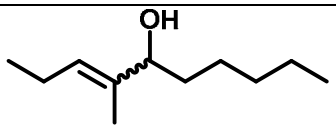
Current regulation: Annex III, part 1, n° 46

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: /

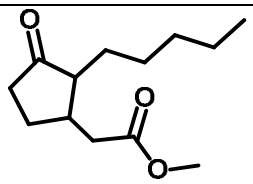
| ***METHYL DECENOL*** |  |
|---|---|
| CAS # 81782-77-6 | |
| EC # 279-815-0 | |
| **4-Methyl-3-decen-5-ol** | |
| | |

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 | |

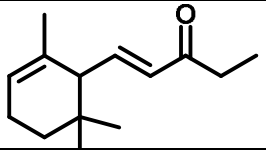
Current regulation: /

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 | |

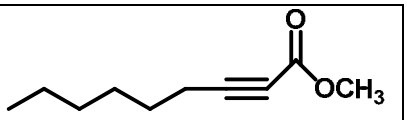
Current regulation: /

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).

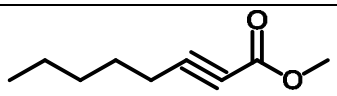
| ***METHYL OCTINE CARBONATE*** |  |
|---|---|
| CAS # 111-80-8 | |
| EC # | |
| **Methyl 2-octynoate** | |
| Methyl 2-Nonynoate, MOC | |

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

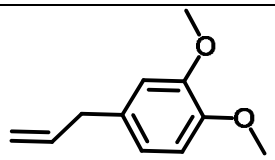
Clinical data:

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- | |

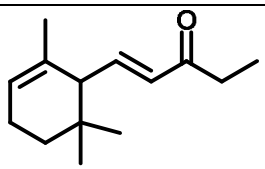
Current regulation: Annex II, 451

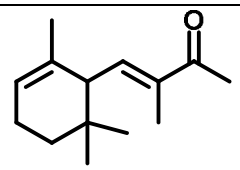
Clinical data:

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** | |

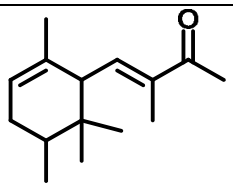
| | |
|---|---|
| 8-Methyl-α-ionone-10-methyl-α-ionone mixt.; Iralia Mixture |  |

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 | |

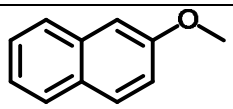
Current regulation: /

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:

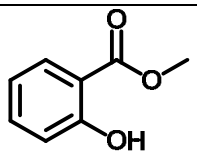
A RIFM review is available (176), citing a (negative) human maximisation test and the study results quoted.

| | |
|---|---|
| ***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:<br>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: /

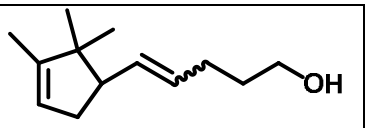
Clinical data:

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").

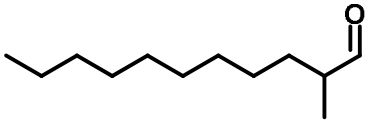
| ***3-METHYL-5-(2,2,3-TRIMETHYL-3-CYCLOPENTENYL)PENT-4-EN-2-OL*** |  |
|---|---|
| CAS # 67801-20-1 | |
| EC # 267-140-4 | |
| **3-Methyl-5-(2,2,3-trimethyl-1-cyclopent-3-enyl)pent-4-en-2-ol** | |
| 3-Methyl-5-(2,2,3-trimethyl-3-cyclopenten-1-yl)-4-penten-2-ol; 3-Methyl-5-(2,2,3-trimethylcyclopent-3-enyl)pent-4-en-2-ol; 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).

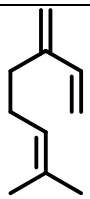
Additional information: /

| ***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 | |

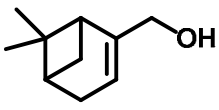
Current regulation: /

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 | |

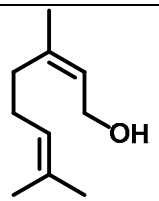
| **(7,7-Dimethyl-4-bicyclo[3.1.1]hept-3-enyl)methanol** |
|---|
| (-)-Pin-2-ene-10-ol; 2-Pinen-10-ol; (6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)methanol; (±)-Myrtenol; 6,6-Dimethyl-2-(hydroxymethyl)bicyclo[3.1.1]hept-2-ene; NSC 408846; α-Pinene-10-ol |

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: /

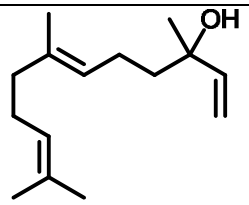
Clinical data:

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.

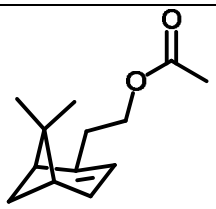
| ***Nerolidol (isomer not specified)*** |  |
|---|---|
| CAS # 7212-44-4 | |
| EC # 230-597-5 | |
| **3,7,11-Trimethyl-1,6,10-odecatrien-3-ol** | |
| Nerolidol; (±)-Nerolidol; FCI 119b; Nerodilol | |

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.

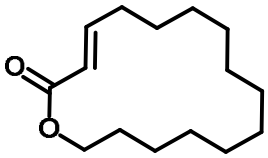
| ***NOPYL ACETATE*** |  |
|---|---|
| CAS # 128-51-8 | |
| EC # 204-891-9 | |
| **2-(7,7-Dimethyl-4-bicyclo[3.1.1]hept-3-enyl)ethyl acetate** | |
| 2-Norpinene-2-ethanol, 6,6-Dimethyl-, acetate; Bicyclo[3.1.1]hept-2-ene-2-ethanol, 6,6-dimethyl-, acetate; 2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl 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).

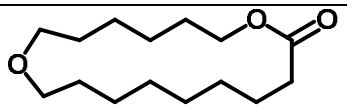
Additional information: /

| ***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 | |

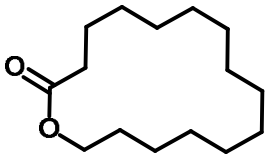
Current regulation: /

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).

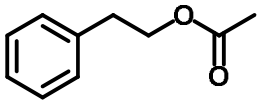
| ***PENTADECALACTONE*** |  |
|---|---|
| CAS # 106-02-5 | |
| EC # 203-354-6 | |
| **1-Oxacyclohexadecan-2-one** | |
| Pentadecanoic acid, 15-hydroxy-, ξ-lactone; 1,15-Pentadecanolide; 15-Hydroxypentadecanoic acid lactone; 15-Pentadecanolide; 15-Pentadodecanolactone; 2-Pentadecalone; CPE 215; Cyclopentadecanolide; 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.

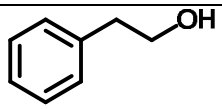
| ***PHENETHYL ACETATE*** |  |
|---|---|
| CAS # 103-45-7 | |
| EC # 203-113-5 | |
| **2-Phenylethyl acetate** | |
| Acetic acid, phenethyl ester ; Phenethyl alcohol, acetate; 2-Phenethyl acetate; 2-Phenylethyl acetate; Benzylcarbinyl acetate; NSC 71927; Phenethyl acetate; Phenylethyl ethanoate; β-Phenethyl 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).

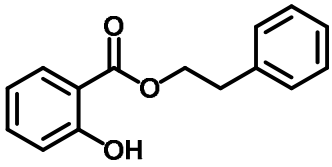
| ***PHENETHYL ALCOHOL*** |  |
|---|---|
| CAS # 60-12-8 | |
| EC # 200-456-2 | |
| **2-Phenylethanol** | |
| Phenethyl alcohol; (2-Hydroxyethyl)benzene; 2-Phenethanol; 2-Phenethyl alcohol; 2-Phenyl-1-ethanol; 2-Phenylethyl alcohol; Benzyl carbinol; Ethanol, 2-phenyl-; NSC 406252; PEA; Phenethanol; Phenethylol; 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).

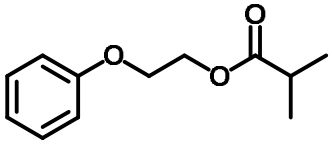
| ***PHENETHYL SALICYLATE*** |  |
|---|---|
| CAS # 87-22-9 | |
| EC # 201-732-5 | |
| **2-Phenylethyl 2-hydroxybenzoate** | |
| Salicylic acid, phenethyl ester; 2-Phenylethyl salicylate; Benzylcarbinyl salicylate; NSC 72035; Phenethyl salicylate | |

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 | |

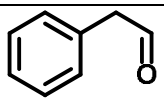
| | |
|---|---|
| 406209; Phenoxyethyl isobutyrate; β-Phenoxyethyl isobutyrate | |

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 | |

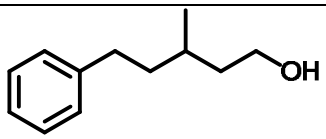
Current regulation: /

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:

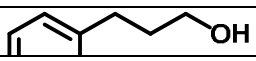
SCCS opinion: 1153/08 - Opinion on "Dermal Sensitization Quantitative Risk Assesment " (QRA: Citral, farnesol and phenylacetaldehyde)

| | |
|---|---|
| ***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*** |  |

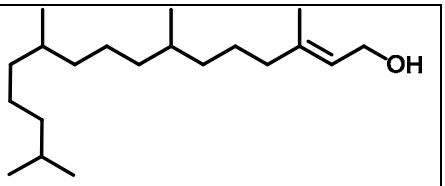
| | |
|---|---|
| CAS # 122-97-4 | |
| EC # 204-587-6 | |
| **3-Phenylpropan-1-ol** | |
| (3-Hydroxypropyl)benzene; 1-Hydroxy-3-phenylpropane; 3-Benzenepropanol; 3-Hydroxy-1-phenylpropane; 3-Phenyl-1-propanol; 3-Phenyl-n-propanol; 3-Phenylpropanol; 3-Phenylpropyl alcohol; Dihydrocinnamyl alcohol; Hydrocinnamic 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: …

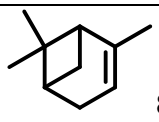
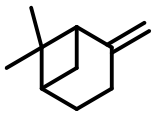
| | |
|---|---|
| ***PHYTOL*** |   |
| CAS # 150-86-7 | |
| EC # 205-776-6 | |
| **(E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol** | |
| Phytol; (7R,11R,2E)-Phytol; (E)-Phytol; (E,R,R)-Phytol; 3,7,11,15-Tetramethylhexadec-2-en-1-ol; trans-Phytol | |

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)<br>**6,6-Dimethyl- 2-methylenebicyclo[3.1.1]heptane** (127-91-3) | |

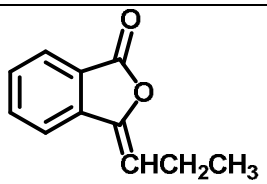
| | |
|---|---|
| **80-56-8**: 2-Pinene; (±)-2-Pinene; (±)-α-Pinene; Acintene A; NSC 7727; PC 500; PC 500 (terpene); Sylvapine A; α-Pinene<br>**127-91-3**: 2(10)-Pinene ; (±)-2(10)-Pinene; (±)-6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane; (±)-β-Pinene; 6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane; NSC 21447; NSC 406265; NSC 59190; Nopinen; Nopinene; PC 600; PC 600 (pesticide); Pseudopinen; Pseudopinene; Terebenthene; β-Pinene | |
| Current regulation: Annex III, part 1, n° 130 (Peroxide value less than 10 mmoles/L in substance) | |

Clinical data:

In 63 patients positive to the FM I, 2 had a positive PT reaction to beta-pinene (and none to alpha-pinene 5% pet.), 1% pet., in the Santucci 1987 study (28). A clinical series from Portugal, addressing contact allergy to oil of turpentine diagnosed in 30 patients, used a series with pure terpenes. A total of 17 of 30 patients reacted positively to alpha-pinene, and 2 to beta-pinene (189). In a series of 24 patients with occupational contact dermatitis from the pottery industry, Lear at al. found 14 to be sensitised to "Indonesian oil of turpentine" and 8 to alpha-pinene (190).

A case report from Zacher and Ippen on 2 patients with allergic contact dermatitis due to bergamot oil (191) describes positive patch test reactions to alpha-pinene and beta-pinene in one, a worker in a perfume factory.

Additional information: /

| | |
|---|---|
| ***PROPYLIDENE PHTHALIDE*** |  |
| CAS # 17369-59-4 | |
| EC # 241-402-8 | |
| **3-Propylidene-2-benzofuran-1-one** | |
| 3-Propylidene-1(3H)-isobenzofuranone; 3-Propylidenephthalide; Celeriax; Propylidenephthalide | |

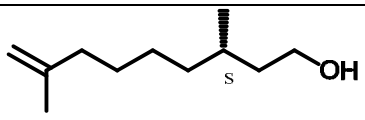


Current regulation: Annex III, part 1, n° 175

Clinical data:

In the Malten 1984 study, 2.6% of 182 patients displayed a positive PT reaction to ethyl acrylate 1% pet. (24). In this paper, "3/25" positive results in human maximisation tests are listed.

Additional information: /

| | |
|---|---|
| ***RHODINOL*** |  |
| CAS # 6812-78-8 | |
| EC # 229-887-4 | |
| **(3S)-3,7-Dimethyloct-7-en-1-ol** | |
| Rhodinol; (-)-Rhodinol; α-citronellol; (-)-α-Citronellol; (S)- | |

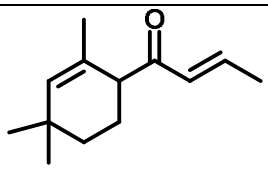
| α-Citronellol | |
|---|---|

Current regulation: /

Clinical data: / (see below)

Additional information:

A RIFM review exists citing a positive HRIPT with several cases of sensitisation, 5 of these proven upon re-challenge, and a negative human maximisation test (192).In a previous RIFM review (128), a Japanese clinical study (source not accessible) is cited: "In patch tests using cosmetics ingredients and fragrance materials on patients with eczema and dermatitis, 5% rhodinol (vehicle not specified) produced one sensitization reaction in 202 patients (Itoh et al., 1988[14])"

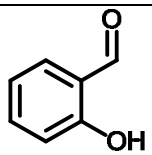
| ***trans-ROSE KETONE-5*** |  |
|---|---|
| CAS # 39872-57-6 | |
| EC # 254-663-8 | |
| **(2E)-1-(2,4,4-Trimethylcyclohex-2-en-1-yl)but-2-en-1-one** | |
| alpha-Isodamascone; trans-2,4,4-Trimethyl-1-crotonyl-2-cyclohexene; (E)-1-(2,4,4-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one | |

Current regulation: Annex III, part 1, n° 159 (max. conc. 0.02%)

Clinical data: /

Additional information:

A RIFM review is available (193) quoting 2 HRIPT studies: one with 0.2% concentration in DEP in 103 volunteers, and negative result, one with 2% concentration, sensitising 2 of 22 volunteers.

| ***SALICYLALDEHYDE*** |  |
|---|---|
| CAS # 90-02-8 | |
| EC # 201-961-0 | |
| **2-Hydroxybenzaldehyde** | |
| Salicylaldehyde; 2-Formylphenol; NSC 112278; NSC 49178; NSC 83559; NSC 83560; NSC 83561; NSC 83562; NSC 97202; Salicylal; Salicylic aldehyde; o-Formylphenol; o-Hydroxybenzaldehyde | |

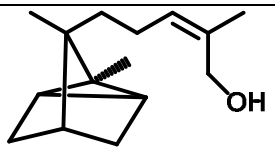
Current regulation: /

Clinical data:

[14] Itoh M., Hosono K., Kantoh H., Kinoshita M., Yamada K., Kurosaka R. and Nishimura M. (1988) Patch test results with cosmetic ingredients conducted between 1978-1986. *Nippon Koshohen Kagakkaishi* 12 (1), 27-41.

In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 1 positive reaction to salicylaldehyde was observed (3). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=1 (0.1%) positive reaction to salicylaldehyde 2% pet. (22). The IVDK 2010 study, 0.48% (95% CI: 0.18 – 0.79%; percentages standardised for age and sex) of 2729 patients PTed reacted to the compound (7). An earlier study by Bruze and Zimerson points to possible cross-reactivity between salicylaldehyde and "simple methylol phenols" occurring in synthetic resins based on phenol and formaldehyde (194). Among 24 patients sensitised to resorcinol by application of a wart remover, 2 positive reactions to salicylaldehyde were observed (195).

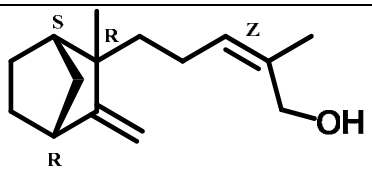
Additional information: Along with other derivates of salicylic acid, salicylaldehyde is found in the bark of several trees, such as willow or aspen, and can cause allergic contact dermatitis by this exposure (196).

| ***alpha-SANTALOL*** |  |
|---|---|
| CAS # 115-71-9 | |
| EC # 204-102-8 | |
| **(R Z)- 5-(2,3-dimethyltricyclo[2.2.1.02,6]hept-3-yl)-2-methylPent-2-en-1-ol** | |
| 2-Penten-1-ol, 5-(2,3-dimethyltricyclo[2.2.1.02,6]hept-3-yl)-2-methyl-, [R(Z)]-; 2-Penten-1-ol, 5-(2,3-dimethyltricyclo[2.2.1.02,6]hept-3-yl)-2-methyl-, stereoisomer; α-Santalol; Tricyclo[2.2.1.02,6]heptane, 2-penten-1-ol deriv.; (+)-(Z)-α-Santalol; (+)-α-Santalol; (Z)-α-Santalol; Sandal; Santalol a; cis-α-Santalol; d-α-Santalol | |

Current regulation: /

Clinical data: / (see beta-santalol)

Additional information: Following a precautionary principle, both isoforms – often not differentiated in reports – are considered as one and considered as established contact allergen in humans.

| ***beta-SANTALOL*** |  |
|---|---|
| CAS # 77-42-9 | |
| EC # 201-027-2 | |
| **(2Z)-2-Methyl-5-[(1S,2R,4R)-2-methyl-3-methylenebicyclo[2.2.1]hept-2-yl]pent-2-en-1-ol** | |
| 2-Methyl-5-(2-methyl-3-methylene-2-norbornyl)-2-penten-1-ol; [1S-[1α,2α(Z),4α]]-2-Methyl-5-(2-methyl-3-methylenebicyclo[2.2.1]hept-2-yl)-2-penten-1-ol; β-Santalol; (-)-(Z)-β-Santalol; (-)-β-Santalol; Santalol b; cis-β-Santalol | |

Current regulation: /

Clinical data:

A RIFM review is available for alpha-santalol (197) and on "santalol" (CAS # 11031-45-1 (198). The former review cites a Japanese study: "Between April 1979 and August 1990, a total of 3123 male and female patients were patch tested to 2% santalol (.alpha. or .beta. not specified) in petrolatum. Reactions were observed in 47/3123 (1.5%) of the patients. The incidence of positive reactions from 1979 to 1990 was 1.5%. The rate of reactions observed was higher during the earlier period of the patch testing than the later stage (Utsumi et al., 1992)[15]." In another Japanese study cited by the RIFM review "... patch tests were conducted with 0.05–0.5% santalol (specified as santalol 1) in a base cream or in 99% ethanol. Patches consisted of a piece of 1 cm2 lint with a 2 cm2 cellophane disc placed on the lint and then covered with a 4 cm2 plaster. Patches were applied to the back, the forearm, and the inside of the upper arm for 24–48 h. Reactions were observed in 15 patients and questionable reactions were observed in 10 patients out of the total 427 participating. A second sample of santalol (specified as santalol 2) was tested on 214 patients. Reactions were observed in three patients and questionable reactions were observed in six patients (Takenaka et al., 1986)[16]." Moreover, "The Mid-Japan Contact Dermatitis Research group (MJDCRG) conducted a 6-year (1976–1981) patch test study on facial dermatoses patients with various fragrance materials. During the year 1979, a total of 327 patients were tested with a mixture of .alpha. and .beta. santalol at concentrations of 10%, 2%, and 1% in white petrolatum. Reactions were observed in 1.5%, 0.6% and 0.6% of the 327 patients tested at concentrations 10%, 2%, and 1%, respectively (MJCDRG, 1984)[17]."

The Goossens 1997 study found 5 of 111 patients positive to "santalol 10% pet." (isoform not specified) – all sensitised to other fragrance allergens as well (23). In the Larsen 2001 study, patch testing with "2-methyl-5-(2,3-dimethyl tricyclo[2.2.1.0(2,6)]hept-3-yl-2 pentenol(.alpha.-form) and 2-methyl-5-(2-methyl-3-methylenebicyclo[2.2.1]hept-3-yl-2-penten-1-ol(beta-form) 5% pet." (no CAS numbers given) yielded a total of 2 positive reactions among the 178 patients with known contact allergy to fragrance ingredients (19).

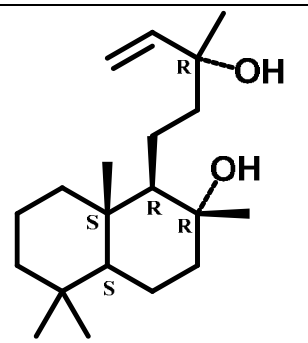
Additional information: "There is no one CAS number for the mixture. The alpha form has a CAS No. 115-71-9 and the beta form is 37172-32-0 (this # is trans-.beta.-santalol). There was no reported use of these materials in the last two IFRA Surveys (8 years total)" (A.M. Api, pers. comm., 2010).

Following a precautionary principle, both isoforms – often not differentiated in reports – are considered as one and considered as established contact allergen in humans

[15] Utsumi, M., Sugai, T., Shoji, A., Watanabe, K., Asoh, S., Hashimoto, Y., 1992. Incidence of positive reactions to sandalwood oil and its related fragrance materials in patch tests and a case of contact allergy to natural and synthetic sandalwood oil in a museum worker. Skin Research 34, 209–213

[16] Takenaka, T., Hasegawa, E., Takenaka, U., Saito, F., Odaka, T., 1986. Fundamental studies of safe compound perfumes for cosmetics Part 1. The primary irritation of compound materials to the skin. Unknown Source, 313–329.

[17] Mid-Japan Contact Dermatitis Research Group, 1984. Determination of suitable concentrations for patch testing of various fragrance materials. A summary of group study conducted over a 6-year period. Journal of Dermatology, 11(1), 31–35.

| ***SCLAREOL*** |  |
|---|---|
| CAS # 515-03-7 | |
| EC # 208-194-0 | |
| **(1R,2R,8aS)-1-[(3R)-3-Hydroxy-3-methylpent-4-enyl]-2,5,5,8a-tetramethyl-3,4,4a,6,7,8-hexahydro-1H-naphthalen-2-ol** | |
| (αR,1R,2R,4aS,8aS)-α-Ethenyldecahydro-2-hydroxy-α,2,5,5,8a-pentamethyl-1-naphthalenepropanol; [1R-[1α(R*),2β,4aβ,8aα]] - α-ethenyldecahydro-2-hydroxy-α,2,5,5,8a-pentamethyl-1 Naphthalenepropanol; (13R)-Labd-14-ene-8,13-diol; Sclareol; (-)-Sclareol; [1R-[1.alpha.(R*),2.beta.,4a.beta.,8a.alpha.]]-2-hydroxy-.alpha.,2,5,5,8a-pentamethyl-.alpha.-vinyldecahydronaphthalene-1-propan-1-ol | |

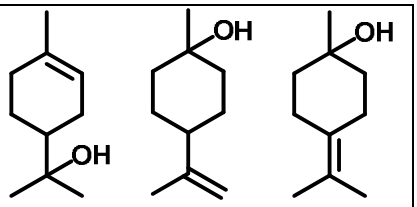
Current regulation: /

Clinical data: /

Additional information:

An older RIFM review exists (128), reporting several human maximisation tests with different samples of sclareol, yielding partly positive, partly negative results. A more recent RIFM review is available (199), citing no clincial data, but several maximisation studies, one of which was positive in a few volunteers, which was apparently due to an impurity.

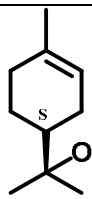
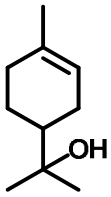
0986/06 - Opinion on Sclareol (sensitisation only) (http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_056.pdf)

| ***TERPINEOL*** |  |
|---|---|
| CAS # 8000-41-7 | |
| EC # 232-268-1 | |
| **Mixtures of isomers** | |
| Terpineol 318, mixture of terpineol isomers alfa, beta, gamma | alfa beta gamma |

Current regulation: /

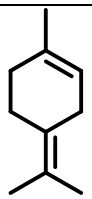
Clinical data:
A RIFM review is available (200), citing negative human induction studies and one clinical study "Takenaka 1986", finding 4 of 312 patients with 0.05% to 0.5% terpineol in a cream base and in ethanol, resp., and 2 negative clinical studies of limited size. In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% terpineol in pet., tested in 100 consecutive patients in Belfast, were observed (15).

Additional information: It is a "top 100" substance (IFRA, pers. comm. 2010).

| | |
|---|---|
| ***alpha-TERPINEOL*** |  10482-56-1 |
| CAS # 10482-56-1 / 98-55-5 | |
| EC # 233-986-8 / 202-680-6 | |
| **2-[(1S)-4-Methyl-1-cyclohex-3-enyl]propan-2-ol** (10482-56-1)<br>**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<br>**98-55-5**: p-Menth-1-en-8-ol; (±)-α-Terpineol; 1,1-Dimethyl-1-(4-methylcyclohex-3-enyl)methanol; 1-p-Menthen-8-ol; 2-(4-Methyl-3-cyclohexenyl)-2-propanol; 4-(2-Hydroxy-2-propyl)-1-methylcyclohexene; 8-Hydroxy-p-menth-1-ene; NSC 21449; NSC 403665; PC 593; Pine Oil 593; Terpineol 350; dl-α-Terpineol; α,α,4-Trimethyl-3-cyclohexene-1-methanol; α-Terpineol |  98-55-5 |
| Current regulation: / | |

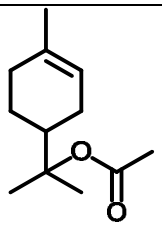
Clinical data:
A RIFM review is available (201) specifically on (-)-alpha-terpineol stating that "no data is available" regarding skin sensitisation. Another RIFM review is available on alpha-terpineol (202). In the Frosch 2002 b study, 1 of 1606 consecutive patients showed a positive reaction, but 11 patients doubtful reactions to alpha-terpineol (5% pet.) (17). The DeGroot 1985 study identified no positive reactions among 179 patients using a 15% PT preparation of terpineol (mixed isomers) (25). In 63 patients positive to the FM I, 2 had a positive PT reaction to alpha terpineol, 5% pet., in the Santucci 1987 study (28). A clinical series from Portugal, addressing contact allergy to oil of turpentine diagnosed in 30 patients, used a series with pure terpenes. A total of 3 of 30 patients reacted positively to alpha-terpineol (189)

Additional information: see also terpineol (mixture of isomers). Comments on turpentine under pinene.

| | |
|---|---|
| ***Terpinolene*** |  |
| CAS # 586-62-9 | |
| EC # 209-578-0 | |
| **1-Methyl-4-propan-2-ylidenecyclohexene** | |
| p-Mentha-1,4(8)-diene; 1-Methyl-4-(1-methylethylidene)-cyclohexene; 4-Isopropylidene-1-methylcyclohexene; Isoterpinene; Nofmer TP; Terpinolen; Terpinolene; α-Terpinolene; δ-Terpinene | |
| Current regulation: Annex III, part 1, n° 133 (Peroxide value less than 10 mmoles/L in substance) | |

Clinical data:
A 49-year-old machine cleaner developed occupational contact dermatitis due to the cleaner, which gave a positive patch test result at 1:10 000 in water. Of the ingredients identified by chromatography, only .delta.-3-carene and terpinolene, tested 5% pet.,

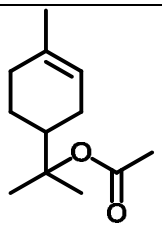
| |
|---|
| gave a positive result (negative in 10 controls) (203). Eleven patients sensitised to tea tree oil showed positive reactions to alpha-terpinene, terpinolene and ascaridol (204).<br><br>Additional information: It is a "top 100" substance (IFRA, pers. comm. 2010) |

| ***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)

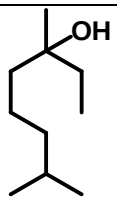
| ***alpha-TERPINYL ACETATE*** |  |
|---|---|
| CAS # 80-26-2 | |
| EC # 201-265-7 | |
| **2-(4-Methyl-1-cyclohex-3-enyl)propan-2-yl acetate** | |
| 3-Cyclohexene-1-methanol, α,α,4-trimethyl-, acetate; p-Menth-1-en-8-ol, acetate; (±)-α-Terpineol acetate; (±)-α-Terpinyl acetate; 2-(4-Methyl-3-cyclohexen-1-yl)-2-propyl 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)

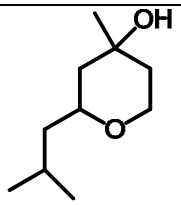
| Tetrahydrolinalool |  |
|---|---|
| CAS # 78-69-3 | |
| EC # 201-133-9 | |
| **3,7-Dimethyloctan-3-ol** | |
| 2,6-Dimethyl-6-octanol; 3,7-Dimethyloctan-3-ol; Linalool tetrahydride; NSC 128151; Tetrahydrolinalool | |

Current regulation: /

Clinical data:
/

Additional information: It is a "top 100" substance (IFRA, pers. comm. 2010). A RIFM

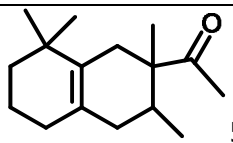
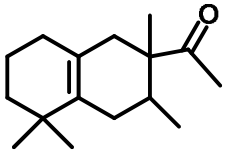
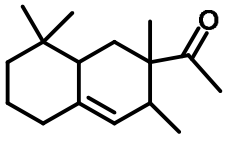
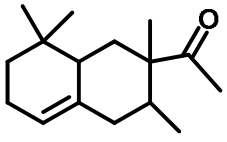
review is available (205) quoting 1 negative human maximisation test.

| ***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).

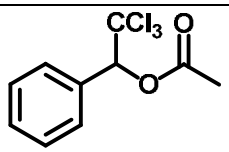
| ***TETRAMETHYL ACETYLOCTAHYDRONAPHTHALENES*** |  54464-57-2 |
|---|---|
| CAS # 54464-57-2 / 54464-59-4 / 68155-66-8 / 68155-67-9 | |
| EC # 259-174-3 / 259-175-9 / 268-978-3 / 268-979-9 | |
| **1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)-ethanone** (54464-57-2)<br>**1-(1,2,3,4,5,6,7,8-Octahydro-2,3,5,5-tetramethyl-2-naphthalenyl)-ethanone** (54464-59-4)<br>**1-(1,2,3,5,6,7,8,8a-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)-ethanone** (68155-66-8)<br>**1-(1,2,3,4,6,7,8,8a-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)-ethanone** (68155-67-9) |  54464-59-4<br>  68155-66-8<br>  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).

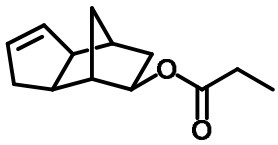
It is a "top 100" substance (IFRA, pers. comm. 2010)

| ***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;<br>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).

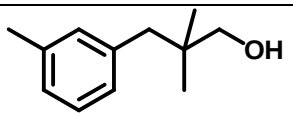
| ***3-(5,5,6-TRIMETHYLBICYCLO[2.2.1]HEPT-2-YL)-CYCLOHEXAN-1-OL*** | |
|---|---|

| | |
|---|---|
| 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 | |

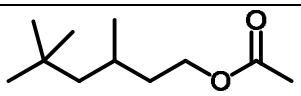


Current regulation: /

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: /

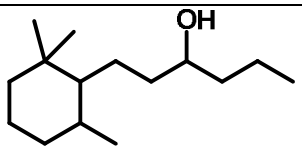
| | |
|---|---|
| ***TRIMETHYLHEXYL ACETATE*** |  |
| CAS # 58430-94-7 | |
| EC # 261-245-9 | |
| **3,5,5-Trimethylhexyl acetate** | |
| 1-Hexanol, 3,5,5-trimethyl-, acetate; Vanoris; neononyl acetate | |



Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm. 2010)

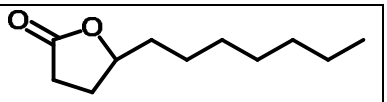
| ***TRIMETHYL-PROPYLCYCLOHEXANEPROPANOL (TMCH)*** |  |
|---|---|
| CAS # 70788-30-6 | |
| EC # 274-892-7 | |
| **1-(2,2,6-Trimethylcyclohexyl)hexan-3-ol** | |
| Other names: 2,2,6-Trimethyl-alpha-propylcyclohexanepropanol (REACH, EINECS); .alpha.-Propyl-2,2,6-trimethyl-cyclohexanepropanol; 6-(2,2,6-Trimethylcyclohexyl)-4-hexanol; 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: /

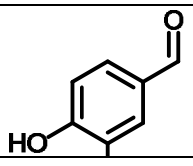
| ***gamma-UNDECALACTONE*** |  |
|---|---|
| CAS # 104-67-6 | |
| EC # 203-225-4 | |
| 5-Heptyltetrahydrofuran-2-one | |
| Undecanoic acid, 4-hydroxy-, γ-lactone; (RS)-γ-Undecalactone; (±)-γ-Undecalactone; 4-Hydroxyundecanoic acid lactone; 4-Undecanolide; 5-Heptyldihydro-2(3H)-furanone; NSC 406421; NSC 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: /

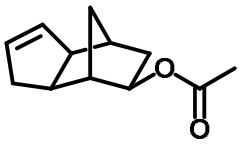
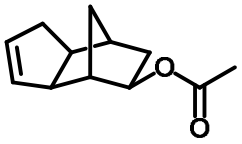
Clinical data:

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)<br>**3a,4,5,6,7,7a-Hexahydro-4,7-methano-1H-inden-5-yl acetat** (5413-60-5) |  <br>2500-83-6<br>  <br>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<br>**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,

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| ***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 |
| $\alpha_D^{20}$ | +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).

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***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.

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|---|---|
| ***ILLICIUM VERUM*** *FRUIT OIL* | *CAS 84650-59-9, 8007-70-3; EC* 283-518-1 |
| *"Anise Oil", Star anise oil* | *(Star anise, Illicium verum, ext. = INCI)* |

Current regulation: /

Clinical data:

In a study involving 100 consecutive patients, Rudzki and Grzywa found (i) a relatively high frequency of active sensitisation to star anise oil (n=5) tested with 0.5, 1 and 2% concentration (most likely in yellow petrolatum, as the other allergens in this series). Later patch testing with constituents of this essential oil (1%) in 3 patients yielded positive results to anethole in 3 cases, and to alpha-pinene and safrole in the 1 case tested to these substances. 34% of the consecutive patients reacted positively to star anise oil at 1%, which was considered as (marginally) non-irritating PT concentration (234).

Additional information:

ISO 4720:2009 nomenclature: *Illicium verum* Hook. f. Illicium Verum Fruit Oil is an essential oil distilled from the fruits of the Star Anise, Illicium verum, Illiciaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40297, last accessed 2010-01-29). The main component is trans-anethole (86-93%), which can be purified from star anise oil. Main uses are alcoholic beverages, food flavouring and oral care products (34, 39). For Oil of star anise, Chinese type (Illicium verum Hook. f.) an ISO standard exists: ISO 11016:1999.

| | |
|---|---|
| ***JASMINUM GRANDIFLORUM*** *FLOWER EXTRACT* | *CAS 84776-64-7; EC* 283-993-5 *(Jasmine, Jasminum grandiflorum, ext. = INCI)* |
| *Jasmine abs.* | |
| ***JASMINUM OFFICINALE*** *FLOWER OIL* | *CAS 90045-94-6; EC* 289-960-1 *(Jasmine, Jasminum officinale, ext. = INCI)* |
| ***JASMINUM OFFICINALE*** *OIL* | *CAS 8022-96-6; EC /* (Oils, jasmine) INCI: JASMINUM OFFICINALE OIL |

Current regulation: /

Clinical data:

In the Frosch 2002 b study, a total of 1.2% of 1606 consecutive patients had a positive PT to "jasmine absolute", tested 5% in pet. (17). The deGroot 2000 study yielded 13 positive reactions to "jasmine, synthetic" in 1825 consecutively tested patients (12). In the early Larsen 1977 study, 18 of 20 "perfume sensitive patients" reacted to "Jasmin synthetic" 10% pet. (18), while 7 reacted to "Jasmin absolute" (10% pet.) – all of these also positive to the synthetic fragrance. The Sugiura 2000 study set in Nagoya, Japan, yielded 1% positive PT reactions in 1483 patients PTed for suspected cosmetic dermatitis, using 5% pet. as test concentration (14). The Larsen 2001 study in 178 patients with known contact allergy to fragrance ingredients found 16.9% positive reactions to jasmine absolute (10% pet.) (19). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had a positive reaction to Jasmine officinale oil (Jasmine absolute, Egyptian), tested at 2% (13). In the NACDG 2009 study, 1.1% of 4447 patients tested with "Jasmine absolute 2% pet." were found PT-positive (21). The Belsito 2006 study (20) yielded 0.4% positive reactions to "jasmine absolute". The Goossens 1997 study found 5 of 111 patients positive to "jasmine absolute" (10% pet.)– all sensitised to other fragrance allergens (23). In 63 patients positive to the FM I, 13 had positive PT reactions to "jasmine absolute", 2% pet., and 12 to "jasmine synthetic", 2% pet. in the Santucci 1987 study – the amount of concomitant reactivity was not examined (28). Nakayama et al. found 1974 (after (29)) 19 "strong positive" and 25 "weak positive" reactions to "jasmin oil" (unknown test concentration) in 183 patients. The IVDK 2010 c study identified 1.5% positive reactions in 3668 consecutively tested patients and 1.2% positive reactions in 982 patients tested in the context of a special series (30). In a study from Alicante, Spain, 86 selected patients were tested with jasmine absolute, yielding 3 positive reactions, and with "Jasmine synthetic", also resulting in 3 positive reactions (48).

Additional information:

Jasminum Grandiflorum Flower Extract is an extract obtained from the flowers of the Spanish Jasmine, Jasminum grandiflorum L., Oleaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39752, last accessed 2009-11-12).

Jasminum Officinale Flower Oil is an essential oil obtained by molecular distillation of the flowers from the Jasmine, Jasminum officinale L., Oleaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39754, last accessed 2009-11-25).

Jasminum Officinale Oil is the volatile oil obtained from the flowers of the Jasmine, Jasminium officinale L., Oleaceae

(http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=34776, last accessed 2010-01-05); this latter extract is used by Almirall/Hermal/Trolab for the preparation of a PT allergen.

Jasmine absolute is obtained by solvent extraction, via concrete, from the flowers of J. grandiflorum (L.) Aiton from China and India. The main volatile compound is benzyl acetate, however, minor compounds such as indole [120-72-9], cis-jasmone [488-10-8] and methyl jasmonate [1211-29-6] contribute to the typical jasmine fragrance (34). Reported compounds include the following (maximum observed concentration given in parentheses): benzyl acetate (28); benzyl benzoate (24.0); phytyl acetate (9); isophytol (8.5); phytol (7.4); linalool (7.0); eugenol (4.0); squalene (4); indole (3.5); benzyl alcohol (2.5); cis-jasmone (2.5); methyl linolenate (2.0); methyl palmitate (1.4); p-cresol (1.0); cis-3-hexenyl benzoate (1.0); benzyl salicylate (0.4); jasmin lactone (0.9); methyl jasmonate (0.7); isoeugenol (0.4) ((30), also according to (17))

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***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)*

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Current regulation: /

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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.

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***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); $\Delta^3$-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).

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***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.

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***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.

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***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)*

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Current regulation: /

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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*

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|---|---|
| ***THYMUS VULGARIS*** *HERB OIL* | *CAS 84929-51-1, 8007-46-3; EC 284-535-7 (Thyme, Thymus vulgaris, ext.)* |
| *"Thyme oil"* | *INCI: THYMUS VULGARIS / Thyme, Thymus vulgaris, ext.* |

Current regulation: /

Clinical data:

The Rudzki 1976 study found no positive reaction in 200 patients to "thyme" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=4 (4.6%) positive reactions to "thyme" essential oil 2% pet. (27). In 63 patients positive to the FM I, none had a positive PT reaction to thymol, 1% pet., in the Santucci 1987 study (28).

Additional information:

ISO 4720:2009 nomenclature: *Thymus vulgaris* L. *Thymus vulgaris* Herb Oil is an essential oil obtained from the herbs of the Thyme, Thymus vulgaris L., Lamiaceae. It contains 20-40% thymol and carvacrol, cymene, pinene, linalool, bornyl acetate (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41133, last accessed 2010-01-29).

Other species are used for extraction, e.g., Thymus Mastichina (CAS 84837-14-9 ), Thymus Serpillum (CAS 84776-98-7 ), Thymus Zygis (CAS 85085-75-2), according to CosIng. The main constituent is thymol (37-56%) (34). For Oil of thyme containing thymol, Spanish type [Thymus zygis (Loefl.) L.] an ISO standard exists: ISO 14715:2010, for Oil of Spanish wild marjoram (Thymus mastichina L.): ISO 4728:2003.

| | |
|---|---|
| ***TURPENTINE*** *(oil)* | *CAS* 8006-64-2 / 9005-90-7 / 8052-14-0; EC 232-350-7 / 232-688-5 / - |

Current regulation: III/124 ; III/125 ; III/126

Clinical data:

Oil of turpentine has been patch tested in a number of baseline series, i.e., in consecutive patients, although not included in the European Baseline series.

In a series of 24 patients with occupational contact dermatitis from the pottery industry, Lear at al. found 14 to be sensitised to "Indonesian oil of turpentine" and 8 to alpha-pinene (190)

**Table 3.2.2 – 2:** Overview of results with **Oil of turpentine** in patients patch tested for suspected allergic contact dermatitis. If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS.

| Country | Population | Years | No. tested | Crude % positive (95% CI) [§] |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| Lisbon, Portugal (189); virtually no .delta.-3-carene | Consecutive patients | 1979-1983 | 4316 | 2.3 % (1.9 – 2.8) § |
| Birmingham, UK (190) | Potters with occup. hand dermatitis | 6 months; prior to 1996 | 24 | 14 / 24 pos. to "Indonesian turpentine" |
| Austria/Germany (IVDK) (279) | Consecutive patients | 1992-1995 | 27658 | 0.47 % (0.39 – 0.55) § |
| Austria/Germany (IVDK) (280) | Consecutive patients | 1996-2002 | 59478 | Annual prevalences 1.6 to 4.4 % |
| Augsburg/Germany (281) | Population sample | 1998 | 1141 | 1.2% (on population level!) |
| Europe (ESSCA) (273) | Consecutive patients | 2002/03 | 3767 | 1.6 % |
| Austria/Germany/ Switzerland (IVDK) (7) | Consecutive patients | 2005-2008 | 37163 | 1.8 % |

Additional information:

ISO 4720:2009 nomenclature: *Pinus pinaster* Aiton and *Pinus massoniana* Lamb. Turpentine, oil: Any of the volatile predominately terpenic fractions or distillates resulting from the solvent extraction of, gum collection from, or pulping of softwoods. Turpentine is a mixture of terpene hydrocarbons obtained from various species of Pinus http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=41521

The composition of oil of turpentine varies with its origin, in particular, the content of .delta.-3-carene, one of its main allergenic compounds (189, 279). Similarly, the peroxide degree may vary. The main constituents are .alpha.-pinene (50-72%), .beta.-pinene (6-15%), carenes (< 0.1-17%), camphene (up to 1%), dipentene (0.5-5%), along with a number of other substances (279).

It is a "top 200" substance and classified as R43 (IFRA, pers. comm.2010)

***Verbena absolute (Lippia citriodora Kunth.)*** *CAS 8024-12-2, 84961-67-1; EC / )*

Current regulation: Annex III, part 1, n° 206

Clinical data: /

Additional information:

ISO 4720:2009 nomenclature: *Aloysia citriodora* Palau syn. *Lippia citriodora* Kunth syn. *Aloysia triphylla* (L' Hér.) Kuntze. An older RIFM review is available citing several positive human maximisation studies both with "Verbena absolute" and "Verbena oil" (128).

***VETIVERIA ZIZANOIDES*** *ROOT OIL*

*CAS 8016-96-4; EC / (Oils, vetiver) / 84238-29-9; EC* 282-490-8 *(*Vetiveria zizanioides, ext. = INCI*)*

*"Vetiver oil; khas khas oil"*

Current regulation: …

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to "vetiver" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=9 (10.4%) positive reactions to "vetiver" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Vetiveria zizanioides* (L.) Nash. Vetiveria Zizanoides Root Oil is an essential oil distilled from the dried roots of the grass *Vetiveria zizanoides* (L.) Nash *Poaceae* (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41293, last accessed 2010-01-29). Vetiver oil has a high sesquiterpene content. The ketones alpha-vetivone [15764-04-2] (6-12%) and beta-vetivone [18444-79-6] (4-10%), which usually form more than 10% of the oil, as well as khusimol [16223-63-5] (24-36%) and isovelencenol [22387-74-2] (12-24%) are the main constituents (in Bourbon oil, i.e., from Réunion) (34). For Oil of vetiver (Vetiveria zizanioides (L.) Nash) an ISO standard exists: ISO 4716:2002.

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## Annex II - Animal Data

**Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)**

| Substance | | | | | EC3 value * | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **INCI name *(other name)*** | **CAS no.** | **Vehicle (AOO=acetone:olive oil; DEP=diethyl phthalate; DMF=dimethyl formamide; DMSO=dimethyl sulphoxide; EtOH=ethanol; MEK=methyl ethyl ketone)** | **Conc. in vehicle (%, generally w/v)** | **No. animals per dose group** | **%** | **µg/cm²** | **M** | **lowest for the substance (%)** | **Comment (deviation from OECD 429 etc)** | **Reference** |
| | | | | | | | | | | |
| *Allyl phenoxyacetate* | 7493-74-5 | 1:3 EtOH:DEP | 0.5, 1.0, 2.5, 5.0, 10.0 | 4 | 3.1 | 775 | 0.16 | 3.1 | | RIFM, 2007a |
| | | | | | | | | | | |
| Amyl cinnamal | 122-40-7 | 1:3 EtOH:DEP | 1.0, 2.5, 5.0, 10.0, 25.0 | 4 | 7.6 | 1900 | 0.38 | 7.6 | | RIFM, 2006a |
| Amyl cinnamal | 122-40-7 | 4:1 AOO | - | 4 | 10.6 | 2650 | 0.52 | | Elahi gives ref to Basketter et al 1999, but no data on the substance is found. It is not known if Elahi, Aptula and Roberts quote the same experiment | Elahi et al., 2004 |

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| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Amyl cinnamal | 122-40-7 | - | - | - | 11 | 2750 | 0.54 | | Aptula gives ref to Kimber et al 2003, but no LLNA data on the substance is found. It is not known if Elahi, Aptula and Roberts quote the same experiment; original reference is not given. | Aptula et al., 2007 |
| Amyl cinnamal | 122-40-7 | - | - | - | 11 | 2750 | 0.54 | | Original ref not given. | Roberts et al., 2007 |
| | | | | | | | | | | |
| Amylcinnamyl alcohol | 101-85-9 | 1:3 EtOH:DEP | 1.0, 2.5, 5.0, 10.0, 25.0 | 4 | > 25 | >6250 | >1.22 | > 25 | Should have been tested at higher concentrations | RIFM, 2004a |
| | | | | | | | | | | |
| Anise alcohol | 105-13-5 | 1:3 EtOH:DEP | 2.5, 5.0, 10.0, 25.0, 50.0 | 4 | 5.9 | 1475 | 0.43 | 5.9 | | RIFM, 2005a |
| | | | | | | | | | | |
| Benzaldehyde | 100-52-7 | - | - | - | - | - | - | | No data in the ref | Roberts et al., 2007 |
| Benzaldehyde | 100-52-7 | - | - | - | - | - | - | | No data in the ref (poster abstract) | Basketter et al., 2003 |
| | | | | | | | | | | |
| Benzyl alcohol | 100-51-6 | 1:3 EtOH:DEP | 2.5, 5.0, 10.0, 25.0, 50.0 | 4 | > 50 | >12500 | >4.62 | > 50 | Should have been tested at higher concentrations | RIFM, 2005b |
| | | | | | | | | | | |

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| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Benzyl benzoate | 120-51-4 | 1:3 EtOH:DEP | 2.5, 5.0, 10.0, 25.0, 50.0 | 4 | > 50 | >12500 | >2.36 | > 50 | Should have been tested at higher concentrations | RIFM, 2005c |
| | | | | | | | | | | |
| Benzyl cinnamate | 103-41-3 | 1:3 EtOH:DEP | 2.5, 5.0, 10.0, 25.0, 50.0 | 4 | 18.4 | 4600 | 0.77 | 18.4 | | RIFM, 2005d |
| | | | | | | | | | | |
| Benzyl salicylate | 118-58-1 | 1:3 EtOH:DEP | 2.5, 5.0, 10.0, 25.0, 50.0 | 4 | 2.9 | 725 | 0.13 | 2.9 | | RIFM, 2005e |
| | | | | | | | | | | |
| *p-tert-Butyl-dihydrocinnamaldehyde* | 18127-01-0 | 1:3 EtOH:DEP | 1.0, 2.5, 5.0, 10.0, 25.0 | 4 | 4.3 | 1075 | 0.23 | 4.3 | | RIFM, 2007b |
| | | | | | | | | | | |
| Butylphenyl methylpropional (BMHCA) | 80-54-6 | EtOH | 1.0, 3.0, 10.0, 30.0, 50.0 | 4 | 2.9 | 725 | 0.14 | 2.9 | | RIFM, 2001a |
| Butylphenyl methylpropional (BMHCA) | 80-54-6 | DEP | 1.0, 3.0, 10.0, 30.0, 50.0 | 4 | 4.1 | 1025 | 0.20 | | | RIFM, 2001b |
| Butylphenyl methylpropional (BMHCA) | 80-54-6 | 1:3 EtOH:DEP | 0.3, 1.0, 3.0, 10.0, 30.0 | 4 | 13.9 | 3475 | 0.68 | | | RIFM, 2001c |
| Butylphenyl methylpropional (BMHCA) | 80-54-6 | 1:3 DEP:EtOH | 0.3, 1.0, 3.0, 10.0, 30.0 | 4 | 8.8 | 2200 | 0.43 | | | RIFM, 2001d |
| Butylphenyl methylpropional (BMHCA) | 80-54-6 | 4:1 AOO | 1.0, 2.5, 5.0, 10.0, 25.0 | 4 | 16.8 | 4200 | 0.82 | | | RIFM, 2001e |

**Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Butylphenyl methylpropional (BMHCA) | 80-54-6 | 4:1 AOO | 1, 2.5, 10, 25, 50 | 4 | 18.7 | 4675 | 0.92 | | | Basketter et al., 2001 |
| | | | | | | | | | | |
| Camellia sinensis leaf *Tea Leaf Absolute* | 84650-60-2 | DMF | 0.5, 1.0, 2.5, 5.0, 10.0 | 4 | > 5.0 | >1250 | N/a | > 5.0 | Should have been tested at higher concentrations | RIFM, 2005m |
| | | | | | | | | | | |
| Cananga odorata leaf / flower oil *Ylang Ylang Extra* | 8006-81-3 | 1:3 EtOH:DEP | 0.5, 1.0, 2.5, 5.0, 10.0 | 4 | 6.8 | 1700 | N/a | 6.8 | | RIFM, 2007f |
| | | | | | | | | | | |
| Carvone | 6485-40-1 | 1:3 EtOH:DEP | 2.5, 5.0, 10.0, 25.0, 50.0 | 4 | 10.7 | 2675 | 0.71 | | | RIFM, 2007c |
| Carvone | 6485-40-1 | 1:3 EtOH:DEP | 2.5, 5.0, 10.0, 25.0, 50.0 | 4 | 5.7 | 1425 | 0.38 | 5.7 | | RIFM, 2007d |
| Carvone | 6485-40-1 | 4:1 AOO | 6.0, 12, 20 | 4 | 13 | 3250 | 0.86 | | | Nilsson et al., 2005 |
| | | | | | | | | | | |
| Cinnamal | 104-55-2 | 3:1 EtOH:DEP | 0.1, 0.3, 1.0, 3.0, 10.0 | 4 | 0.2 | 50 | 0.015 | 0.2 | | RIFM, 2003a |
| Cinnamal | 104-55-2 | 0.1% α-tocopherol in 3:1 EtOH:DEP | 0.1, 0.3, 1.0, 3.0, 10.0 | 4 | 0.2 | 50 | 0.015 | | | RIFM, 2003b |
| Cinnamal | 104-55-2 | 2.0% α-tocopherol in 3:1 EtOH:DEP | 0.1, 0.3, 1.0, 3.0, 10.0 | 4 | 0.6 | 150 | 0.045 | | | RIFM, 2003c |
| Cinnamal | 104-55-2 | 0.3% antioxidant mix (equal parts BHT, tocopherol and eugenol) in 3:1 | 0.1, 0.3, 1.0, 3.0, 10.0 | 4 | 0.7 | 175 | 0.053 | | | RIFM, 2003d |

**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 |
| | | | | | | | | | | |

**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)**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 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 |

**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)**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 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 |

**Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Isoeugenol | 97-54-1 | propylene glycol | 0.5, 1.0, 2.5, 5.0, 10.0 | 4 | 2.5 | 625 | 0.15 | | | Wright et al., 2001 |
| Isoeugenol | 97-54-1 | DMSO | 0.5, 1.0, 2.5, 5.0, 10.0 | 4 | 0.9 | 225 | 0.055 | | | Wright et al., 2001 |
| Isoeugenol | 97-54-1 | 90:10 EtOH:water | 0.5, 1.0, 2.5, 5.0, 10.0 | 4 | 1.8 | 450 | 0.11 | | | Wright et al., 2001 |
| Isoeugenol | 97-54-1 | 50:50 EtOH:water | 0.5, 1.0, 2.5, 5.0, 10.0 | 4 | 4.9 | 1225 | 0.30 | | | Wright et al., 2001 |
| | | | | | | | | | | |
| *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 |
| | | | | | | | | | | |

**Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Methyl 2-octynoate | 111-12-6 | 1:3 EtOH:DEP | 0.5, 1.0, 2.0, 5.0, 10.0 | 4 | < 0.5 | < 125 | <0.032 | < 0.5 | Should also have been tested at lower concentrations | RIFM, 2005k |
| | | | | | | | | | | |
| *2-Methoxy-4-methylphenol* | 93-51-6 | - | - | - | 5.8 | 1450 | 0.42 | 5.8 | | Roberts et al., 2007 |
| | | | | | | | | | | |
| *1-Octen-3-yl acetate* | 2442-10-6 | 1:3 EtOH:DEP | 7.5, 15.0, 30.0 | 5 | > 30 | > 7500 | >1.76 | > 30 | Should have been tested at higher concentrations | RIFM, 2004g |
| | | | | | | | | | | |
| Perillaldehyde *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 |

**Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Phenylacetaldehyde* | 122-78-1 | - | - | - | - | - | - | | No data in the ref (poster abstract) | Basketter et al., 2003 |
| | | | | | | | | | | |
| *3-Propylidenephthalide* | 17369-59-4 | 4:1 AOO | 5, 10, 20 | 4 or 5 | 3.7 | 925 | 0.21 | 3.7 | Should also have been tested at lower concentrations | Gerberick et al., 2004 |
| | | | | | | | | | | |
| Tetramethyl acetyloctahydronaphthalenes (OTNE) | 54464-57-2 | 1:3 EtOH:DEP | 2.5, 5.0, 10.0, 25.0, 50.0 | 4 | 25.14 | 6285 | 1.07 | 25.14 | | RIFM, 2005l |
| | | | | | | | | | | |
| Trimethylbenzenepropanol *Majantol* | 103694-68-4 | 4:1 AOO | 3.0, 10.0, 30.0 | 4 | ~30 | ~7500 | ~1.68 | 30 | Should have been tested at higher concentrations | RIFM, 2002d |
| | | | | | | | | | | |
| Vanillin | 121-33-5 | 4:1 AOO | 2.5, 5, 10, 25, 50 | 4 | >50.0 | >1250 | >3.3 | >50.0 | | Basketter et al., 2001 |
| | | | | | | | | | | |

* source of EC3 value value: % given in the RIFM report or references; µg/cm2 given in the RIFM report and RIFM poster; M calculated by SCCS working group

**material with low levels of oxidation according to RIFM, 2009

- = no data given;  A216

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## Annex III - Tabular summary of dose-elicitation studies in sensitised patients

## Contents

Chloroatranol ..... 316
Cinnamal ..... 318
Hydroxycitronellal ..... 321
Hydroxyisohexyl 3-cyclohexenecarboxaldehyde (HICC) ..... 323
Isoeugenol ..... 329
References ..... 333

### 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<br>=0.0004 µg/$cm^2$ |
| PT ED50% (95% CI) | 0.15 (0.077-0.295) ppm<br>=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).

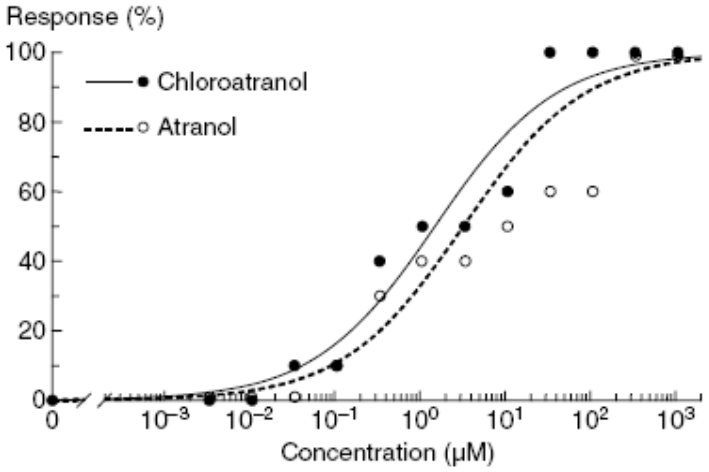



*Fig. 1.* Observed response rates and fitted parallel logistic dose–response curves for atranol and chloroatranol in equimolar concentrations at patch testing. The response was dichotomized and any reaction other than zero was classified as positive.

### 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%<br>= 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<br>(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)<br>average cases: 263 mg/application controls: only range given |
| dose | Part one: Step 1: 0.032% Step 2: 0.1% Step: 0.32%<br>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<br>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)<br>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/$cm^2$<br>29 (7-69) ppm |
| PT ED50% (95% CI) | 20 µg/$cm^2$<br>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/$cm^2$<br>25 ppm (0.69-120) |
| PT ED50% (95% CI) | 18.3 µg/$cm^2$<br>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<br>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.<br>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<br>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.

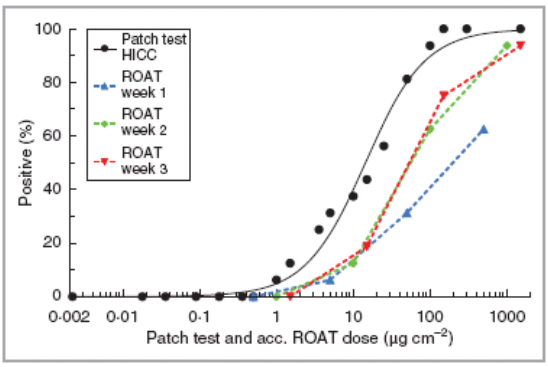



Fig 3. The fitted dose–response curve for the patch test (n = 16) and the 1-week, the 2-week 3-week accumulated ROAT doses.

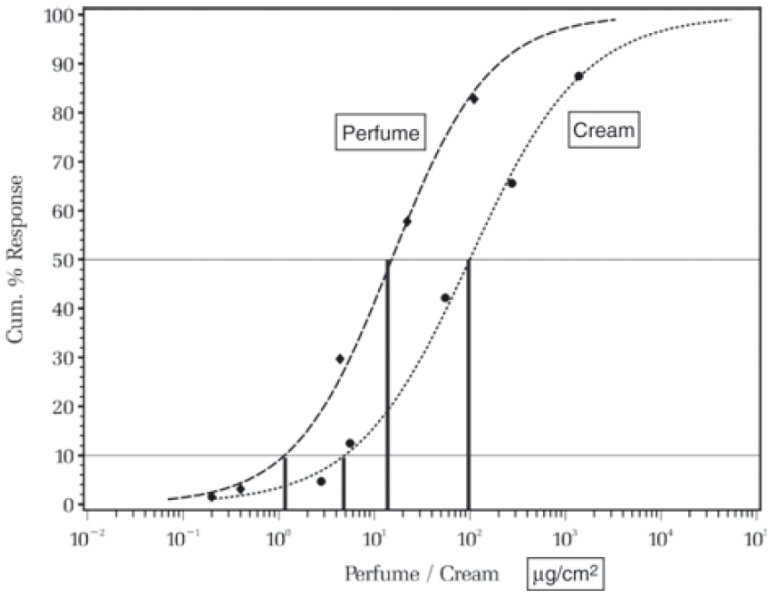
**Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)**

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<br>5.6 µg/cm² in cream<br>55.6 µg/cm² in cream<br>277.8 µg/cm² in cream<br>1388.9 µg/cm² in cream | 0.2 µg/cm² in ethanol<br>0.4 µg/cm² in ethanol<br>4.4 µg/cm² in ethanol<br>22.2 µg/cm² in ethanol<br>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%<br>55.6 µg/cm²: 42.2%<br>277.8 µg/cm²: 65.6%<br>1388.9 µg/cm²: 87.5% | 0.4 µg/cm²: 3.1%<br>4.4 µg/cm²: 29.7%<br>22.2 µg/cm²: 57.8%<br>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<br>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%<br>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<br>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)<br>average cases: 266 mg/application controls: only range given |
| dose | Part 1: Step 1:0.02% Step 2: 0.063% Step 3:0.2%<br>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/ $cm^2$<br>(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<br>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 Assessment of Citrus-Derived Peel Oils as Used in Cosmetics

| | |
|---|---|
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The 2014 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D., Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is Lillian J. Gill, D.P.A. This report was prepared by Christina Burnett, Senior Scientific Analyst/Writer and Monice Fiume, Assistant Director/Senior Scientific Analyst.

**Cosmetic Ingredient Review**

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## ABSTRACT

*The CIR Expert Panel assessed the safety of 14 Citrus-derived peel oil ingredients and concluded that these ingredients are safe for use in cosmetic products when finished products, excluding rinse-off products, do not contain more than 0.0015% (15 ppm) 5-methoxypsoralen (5-MOP), and when formulated to be non-sensitizing and non-irritating. The citrus-derived peel oil ingredients are most frequently reported to function in cosmetics as fragrances and/or skin conditioning agents. The Panel reviewed the available animal and clinical data to determine the safety of these ingredients. Because final product formulations may contain multiple botanicals, each containing similar constituents of concern, formulators are advised to be aware of these constituents and to avoid reaching levels that may be hazardous to consumers. Industry should use good manufacturing practices to limit impurities that could be present in botanical ingredients.*


## INTRODUCTION

Citrus-derived peel oils are widely used as cosmetic ingredients, and are most frequently reported to function in cosmetics as fragrances and/or skin conditioning agents (Table 1). This report assesses the safety of the following 14 citrus-derived peel oils:

Citrus Aurantifolia (Lime) Peel Oil
Citrus Aurantium Amara (Bitter Orange) Peel Oil
Citrus Aurantium Currassuviensis Peel Oil
Citrus Aurantium Dulcis (Orange) Peel Oil
Citrus Clementina Peel Oil
Citrus Grandis (Grapefruit) Peel Oil
Citrus Iyo Peel Oil
Citrus Junos Peel Oil
Citrus Limon (Lemon) Peel Oil
Citrus Medica Vulgaris Peel Oil
Citrus Nobilis (Mandarin Orange) Peel Oil
Citrus Reticulata (Tangerine) Peel Oil
Citrus Tachibana/Reticulata Peel Oil
Citrus Tangerina (Tangerine) Peel Oil

The citrus ingredients in this assessment are found in foods, and daily exposure from food use would result in much larger systemic exposures than those from use in cosmetic products. Additionally, essential oils, oleoresins (solvent-free), and natural extracts (including distillates) derived from some citrus fruits are generally recognized as safe (GRAS) for their intended use in foods for human and animal consumption according to the Food and Drug Administration (FDA). Volatile oils of limes, lemons, grapefruits, bitter oranges, oranges, and tangerines are described as flavoring agents in the United States Pharmacopeia (USP) Food Chemicals Codex.[1] Thus, the systemic toxicity potential of citrus-derived peel oils via oral exposure is not addressed further in this report. The primary focus of the safety assessment of these citrus ingredients as used in cosmetics is on the potential for irritation and sensitization from dermal exposure.

The CIR does not review ingredients that function only as fragrance ingredients because, as fragrances, the safety of these ingredients is evaluated by the Research Institute for Fragrance Materials (RIFM). Three of the citrus-derived peel oils in this report function only as fragrance ingredients, according to the *International Cosmetic Ingredient Dictionary and Handbook* (see Table 2).[2] However, RIFM has not confirmed that these three ingredients function only as fragrances and therefore within its purview, thus CIR is reviewing the safety of these ingredients.

Botanicals such as citrus contain hundreds of constituents, some of which have the potential to cause toxic effects. For example, bergapten (aka 5-methoxypsoralen or 5-MOP) is a naturally-occurring phototoxic furanocoumarin (psoralen) in citrus peel oils. In this assessment, CIR is reviewing information available to evaluate the potential toxicity of each of the citrus-derived peel oils as a whole, complex substance. Except for specific constituents of concern, CIR is not reviewing information that may be available to assess the potential toxicity of the individual constituents of which the citrus-derived ingredients are composed. CIR requested information on the concentrations (including ranges, means, upper 95 percent confidence limits, detection limits, etc.) of individual constituents in citrus-derived peel oils used in cosmetics to facilitate the safety assessment of these ingredients. Such information on constituents that have been identified as constituents of concern by the Panel in previous safety assessments, or by other recognized scientific expert review bodies, is especially important.

Some toxicological data on lemon oil and sweet orange oil (synonyms: lemon, ext. and orange, sweet, ext., respectively) reviewed in this safety assessment were obtained from robust summaries of data submitted to the European Chemical Agency (ECHA) by companies as part of the REACH chemical registration process. These data are available on the ECHA website.[3,4]

Note: In many of the published studies included in this assessment, the information provided is not sufficient to determine how well the substance being tested represents the cosmetic ingredient. In this safety assessment, if a substance tested in a study is not clearly a cosmetic ingredient, because of lack of information on the genus and species from which the substance was derived and/or the method of extraction used, the test substance will be referred to by a common name (e.g. lime oil). If the substance is clearly a cosmetic ingredient, the International Nomenclature of Cosmetic Ingredients (INCI) name will be used (e.g. "citrus aurantifolia (lime) peel oil"). In some instances, the part of the plant from which the oil was expressed is not known but, based on the method of manufacture, the oil could have been expressed from the peels of the citrus fruit. Additionally, some inconsistencies were noted in both taxonomic and INCI naming conventions. For example, this report includes the sweet orange ingredient described as citrus aurantium dulcis (orange) peel oil in the *International Cosmetic Ingredient Dictionary and Handbook*.[2] In contrast, most of the published literature and FDA refer to this ingredient as citrus sinensis (sweet orange) peel oil. Another example of a naming inconsistency is citrus grandis (grapefruit) peel oil; *Citrus grandis* is generally considered a name for a pummelo, and is more commonly called *Citrus maxima*. The INCI Committee of the Personal Care Products Council (Council) is working to correct some of these errors.

## CHEMISTRY

### Definition and General Characterization

The definitions and functions of the citrus-derived peel oils included in this report are provided in Table 1. In some cases, the definition provides insight on the method(s) of manufacture. It should be noted that essential oils are hydrophobic, liquid, volatile aroma compounds from plants. These are typically mixtures of small molecules, but their chemical structures can vary widely. The volatile nature of essential oils makes them likely to be useful as fragrances, but does not preclude other functions for them in cosmetics.

There are numerous *Citrus* varieties distributed throughout the world; most citrus fruits are grown in the temperate and tropical zones in both the northern and southern hemispheres.[5] Citrus can be propagated and new varieties can be produced by cultivating asexual nuclear or chance seedlings or by crossing or mutation.

### Physical and Chemical Properties

Physical and chemical properties of the citrus-derived peel oils are provided in Table 3.

### Method of Manufacturing

A suitable antioxidant can be added to citrus oil during preparation.[1]

Bitter orange oil and orange oil are obtained using a cold-pressed extraction method from the fresh peel of the fruit of *Citrus aurantium* and *Citrus sinensis*, respectively.[1] *Citrus grandis* oil[6] and *Citrus junos* oil[7] are also extracted using a cold-press method. Tangerine oil (cold-pressed) is obtained from the peels of the ripe fruit of the Dancy tangerine, *Citrus nobilis* or *Citrus reticulata*, and from some other closely related varieties.[1]

Lemon oil and lime oil, expressed, are produced by pressing the outer rind of the ripe fruit by hand or by machine.[8] More economical processes involve an integrated juice-oil procedure. These oils can also be produced by distillation of expressed oils or direct distillation of fruit; distilling (rectifying) removes terpenes. Steam distillation removes non-volatile furocoumarins.

Citrus iyo oil is produced by removing the peel from the fruit, homogenization of the peel with distilled water, and lyophilization.[9] The oil is purified using vacuum distillation.

Mandarin peel oil, expressed (identified in the literature as *Citrus reticulata*), is prepared by expression of the peels of the ripe fruit of the mandarin orange.[10]

### Constituents/Composition

The citrus-derived peel oils are complex botanicals composed of numerous constituents; there is great variation among citrus species and cultivars because of frequent bud mutations, interspecific and intergeneric hybridization, and apoximis (i.e., one or more of several types of asexual reproduction).[11] The composition of citrus oils will vary based on the location where the plant is grown, the maturity of the plant, and storage conditions.[5] The method of extraction will also affect the composition.

Citrus oils contain large amounts of monoterpene hydrocarbons. Limonene is the constituent present in the greatest amount, often comprising greater than 90% of the oil, and the amount present can vary within the oil; for example, limonene is reported to compose 38.1%-95.8% *C. limon* (lemon) peel essential oils, cold pressed.[12,13] Citrus oils

also contain sesquiterpene hydrocarbons, which are responsible for the characteristic flavors of these oils.[12] Table 4 lists the chemical compositions of cold-pressed,[6,7,11-16] hydrodistilled,[17-21] lyophilization/vacuum distillation,[9] and steam-distilled[22] peel oils sorted by citrus plant species, Table 5 presents the typical levels of 5-MOP found in some of the oils, and Table 6 provides the levels of major coumarins and furocoumarins in lemon and lime oil. Table 7 lists citrus constituents that are established contact allergens, according the European Commission's Scientific Committee on Consumer Safety (SCCS).

## USE

### Cosmetic

Table 8 presents the current product-formulation use data for citrus-derived peel oils. These ingredients are most frequently reported to function as fragrances and/or skin conditioning agents-miscellaneous.[2]

According to information supplied to the FDA by industry as part of the Voluntary Cosmetic Registration Program (VCRP), citrus limon (lemon) peel oil has the most reported uses in cosmetic and personal care products, with a total of 490; more than half of the uses are in leave-on skin care preparations.[23] Citrus aurantium dulcis (orange) peel oil (reported as citrus sinensis (sweet orange) peel oil by the VCRP) has the second greatest number of overall uses reported, with a total of 289; about half of those uses are in leave-on skin care preparations.

In a use concentration survey conducted by the Council, citrus limon (lemon) peel oil had a maximum use concentration range of 0.0001% to 0.5% with 0.5% reported in "other" skin care preparations.[24] Citrus aurantium dulcis (orange) peel oil had a maximum use concentration range of 0.00002% to 29%, with 29% reported in non-coloring hair conditioners.

In some cases, reports of uses were received from the VCRP, but no concentration of use data were provided. For example, citrus junos peel oil is reported to be used in 6 formulations, but no use concentration data were available. In other cases, no reported uses were identified by the VCRP, but a maximum use concentration was provided in the industry survey. For example, citrus grandis (grapefruit) peel oil was not reported in the VCRP database to be in use, but the industry survey indicated that it is used at concentrations up to 0.05%. It should be presumed that citrus grandis (grapefruit) peel oil is used in at least one cosmetic formulation.

Table 9 lists the seven citrus-derived peel oils not indicated to be in use based on the VCRP data and the results of the Council concentration of use survey.

Under the rules governing cosmetic products in the European Union, citrus-derived ingredients must have furocoumarin content below 1 mg/kg in sun-protection products and in bronzing products.[25] The International Fragrance Association (IFRA) has issued standards for citrus oils and other furocoumarin-containing essential oils.[26] Thus, finished products that are applied to the skin, excluding rinse-off products like bath preparations and soaps, must not contain more than 0.0015%, or 15 ppm, 5-MOP. This equates to 0.0075%, or 75 ppm, in a fragrance compound used at 20% in a consumer product that is applied to the skin. If the level of 5-MOP has not been determined, limits specified for individual oils should be observed, and when such oils are used in combination with other phototoxic ingredients, the potential for an additive effect should be considered and use levels should be reduced accordingly. Restrictions for furocoumarin-containing essential oils have been recommended for bitter orange oil expressed, grapefruit oil expressed, lemon oil cold pressed, and lime oil expressed.

An IFRA standard also has been issued for 7-methoxycoumarin, which is prohibited for use in fragrance compounds.[27] Based on established maximum levels of this substance from commercially-available natural sources (like essential oils, extracts and absolutes), exposure to 7-methoxycoumarin from the use of these oils and extracts is regarded to be acceptable if the level of 7-methoxycoumarin in the finished product does not exceed 100 ppm. An example of a maximum concentration based on this standard is 0.1% for lime cold pressed oil.

Additionally, IFRA has set a standard stating that d-, l-, and dl-limonene and natural products containing substantial amounts of it, should only be used when the level of peroxides is kept to the lowest practical level; such products should have a peroxide value of <20 mmol/l.[28] This standard is also cited by the European Commission.[29] The European Union also states that limonene must be included in the list of ingredients when its concentration exceeds 0.001% in leave-on products and 0.01% in rinse-off products.

The IFRA has also set limits on the amounts of some citrus-derived oils in finished products. For leave-on products applied to skin areas exposed to direct sunlight, these limits include: 1.25% bitter orange peel expressed;[30] 4% grapefruit oil expressed;[31] 2% lemon oil cold-pressed;[32] 0.7% lime oil expressed.[33] There are no restrictions for any of these oils in rinse-off products and products that are not applied to the skin. IFRA specified that if combinations of phototoxic fragrance ingredients are used, the use levels must be reduced accordingly, so that the sum of the concentrations of all phototoxic fragrance ingredients, expressed as a percentage of their respective recommended maximum levels, shall not exceed 100% in the consumer product. Additionally, the IFRA general standard described above for 'Citrus oils and other furocoumarins-containing essential oils' must be considered.

### Non-Cosmetic

The essential oils, oleoresins (solvent-free), and natural extractives (including distillates) derived from the following citrus fruits are GRAS for their intended use in foods for human consumption: *Citrus aurantifolia* (lime); *Citrus aurantium* (bitter orange; the flowers and peel); *Citrus limon* (lemon); *Citrus reticulata* (tangerine); *Citrus reticulata blanco* (mandarin); *Citrus sinensis* (orange; the leaf, flowers, and peel) and citrus peels (species not specified) (21CFR182.20). These essential oils, oleoresins (solvent-free), and natural extractives (including distillates) of these citrus fruits are GRAS for their intended use in animal drugs, feeds, and related products (21CFR582.20).

Citrus essential oils are used in the pharmaceutical industry as flavoring agents to mask the unpleasant taste of drugs.[18] *Citrus aurantium* amara (bitter orange) and extracts of its dried fruit and peel have been used in traditional Western medicines and in Chinese and Japanese herbal medicines.[34]

## TOXICOLOGICAL STUDIES

As noted earlier, the citrus ingredients in this assessment are found in foods, and daily exposures from food use would result in a much larger systemic exposure than those from use in cosmetic products. Essential oils, oleoresins (solvent-free), and natural extracts (including distillates) derived from some citrus fruits are GRAS for their intended use in foods for human and animal consumption according to the FDA. Volatile oils of limes, lemons, grapefruits, bitter oranges, oranges, and tangerines are described as flavoring agents in the USP Food Chemicals Codex.[1] Therefore, the systemic toxicity potential of these ingredients is not addressed further in this report. The primary focus of this safety assessment is on the potential for irritation and sensitization from dermal exposure to these citrus ingredients as used in cosmetic products.

### Acute Toxicity

***Dermal – Non-Human***

Lemon Oil

The dermal $LD_{50}$ of lemon oil was greater than 10 g/kg in rabbits.[3] An occlusive patch of undiluted oil was applied to the skin of six animals for 24 h. One animal died during the observation period.

Mandarin Oil – Expressed (*Citrus reticulata*)

The dermal $LD_{50}$ of mandarin peel oil (*Citrus reticulata*) was greater than 5 g/kg in rabbits.[10] An occlusive patch of undiluted oil was applied to the skin of seven animals for 24 h.

Orange Oil

The dermal $LD_{50}$ of sweet orange oil was greater than 5 g/kg in female New Zealand White rabbits.[4] An occlusive patch of undiluted oil was applied to the skin of 10 animals for 24 h. Signs of skin irritation were reported, including moderate redness in 10/10 animals, slight edema in 3/10 animals, and moderate edema in 5/10 animals.

## REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

No published reproductive and developmental studies on citrus-derived peel oils were identified in a literature search for these ingredients, and no unpublished data were submitted.

## GENOTOXICITY

Genotoxicity studies for in vitro assays are summarized in Table 10. No genotoxic effects were observed for lemon oil or sweet orange oil in bacterial reverse mutation assays, mouse lymphoma cell mutation assays, and Chinese hamster chromosome aberration assays.[3,4]

## CARCINOGENICITY

Orange Oil, Lemon Oil, Grapefruit Oil and Lime Oil

Tumor-promoting activity was observed in mouse skin exposed to essential oils of orange (sweet), lemon, grapefruit, or lime.[35] Chemical constituents of these oils were not fully identified in this study, although the terpene and non-terpene fractions were separated. Groups of 10 male and 10 female strain 101 mice received a single application of 9,10-dimethyl-1,2-benzanthracene (DMBA) in acetone (300 µg in 0.2 ml in 4 groups, 225 µg in 0.15 ml in a fifth group). Group 1 was a control group that received no further treatments. Groups 2 through 5 received weekly applications of 0.25 ml of the test substances 3 weeks after the application of DMBA.

By the fifth week, papillomas were observed in Group 3 (lemon oil), Group 4 (grapefruit oil), and Group 5 (lime oil). Papillomas were observed in Group 2 (orange oil) by the 12$^{th}$ week. After 33 weeks, 10/20 mice in the lemon oil and lime oil treatment groups and 13/20 mice in the grapefruit oil and orange oil groups had papillomas. Only 1 mouse in the control group had papillomas after 33 weeks, and the affected site was not the treated skin. Additionally, one female mouse of the lemon oil group developed a sebaceous-gland tumor of the nipple. No malignant skin tumors were observed in the orange oil group: treatment for this group was stopped after 42 weeks. Squamous cell carcinomas of the skin were observed in two mice from the lemon oil group and two mice of the grapefruit oil group between weeks 36 and 55. One malignant skin tumor was observed in the lime oil group at week 34; however, the mouse was found dead and a proper histological examination was not possible. No malignant skin tumors were observed in the control group. Non-dermal tumors during the treatment period were observed in one mouse of the orange oil group (a hemangioma of the subcutaneous tissue starting at week 7) and in one mouse of the grapefruit oil group (a spindle cell sarcoma of the subcutaneous tissues). No tumors of the internal organs were observed. The survival of all the mice, including the controls, in this experiment was poor because of a very high incidence of renal disease.[35]

Orange Oil

Tumor-promoting activity was observed in mouse skin exposed to orange (sweet) oil.[35] In the study, groups of 10 male and 10 female strain 101 mice received a single application of DMBA in acetone (300 µg in 0.15 ml). One group (15 mice of each sex) was a control group that received no further treatments. Two groups received weekly applications of 0.25 ml of 40% orange oil in acetone or 80% orange oil in acetone 3 weeks after the initial application of DMBA. The applications continued for 37 weeks.

Papillomas were observed in both groups treated with orange oil starting on the 12$^{th}$ week. After 33 weeks, 5/10 mice treated with 40% orange oil and 10/10 mice treated with 80% orange oil had papillomas, and at study end, only 1 tumor of each group was found outside of the treated area. Four mice in the control group had papillomas by week 33, but these tumors were outside of the treated area of the skin. Malignant tumors were observed in one mouse of each treatment group, arising from the pre-existing papilloma. Both tumors were squamous-cell carcinomas infiltrating the panniculus muscle. Additionally, tumors of the urethral orifice were observed in 4 female mice of the 40% orange oil group. The survival of all the mice in this experiment was poor due to a very high incidence of renal disease.

In the same study, tumor-promoting activity was observed in mice exposed to undiluted orange oil after pretreating the mice with either dermal or intraperitoneal injections of urethane. The effect was weak compared to the effects observed after DMBA induction.

A similar experiment performed in the same study tested the carcinogenic effects of orange oil without pretreatment with DMBA or urethane. The mice were treated once weekly with 0.25 ml of 40% or 80% orange oil in acetone or undiluted orange oil for 38 (diluted) or 46 (undiluted) weeks. This study found no evidence of direct tumorigenic effects on the treated mouse skin. Urethral orifice tumors were observed in one female mouse of the 40% orange oil group and in one female mouse of the 80% orange oil group. A papilloma was observed on the head of a mouse (outside of the treatment site) that was treated with 80% orange oil.[35]

## IRRITATION AND SENSITIZATION

### Dermal Irritation

Dermal irritation studies are summarized in Table 11. Lemon oil, orange oil, and mandarin peel oil all produced some reaction in irritation studies in animals, but in human subjects, no irritation was observed after topical exposure to lemon oil (up to 20%) or mandarin peel oil (8%).[3,4,10,36]

## Ocular Irritation

Lemon Oil

Lemon oil tested at 5% was not irritating to the eyes of 3 albino rabbits.[3] Each rabbit had 0.1 ml of the test material instilled into the right eye with no further treatment. The left eye served as the control. Eyes were examined every 24 h for 4 days and then again on the 7th day. No corneal opacity or iris congestion was observed. An intense conjunctival irritation involving chemosis and discharge occurred. Treated eyes were normal on the 7th day.

Orange Oil

Orange oil tested undiluted did not induce significant or irreversible damage to the eyes of three male New Zealand White rabbits.[4] The test was performed in accordance with OECD guideline 405. The test material (0.1 ml) was instilled into one eye of each rabbit. The mean scores calculated for the three animals across 3 scoring times were 0.0 for corneal opacity and iris lesions and 1.0 for reddening of the conjunctivae.

## Sensitization

Sensitization studies are presented in Table 12. Mandarin peel oil (8% in petrolatum) was not sensitizing in human maximization tests. In studies of 250 dermatitic patients, less than 2.5% had positive reactions to bitter orange oil, lemon oil, or sweet orange oil tested at 2% in paraffin.[3,4,10,37]

## Phototoxicity and Photosensitization

Phototoxicity and photosensitization studies are presented in Table 13. Phototoxic potential was observed for sweet orange oil and lemon oil in in vitro studies. No phototoxic responses were noted in some animal studies of lime oil, lemon oil, grapefruit oil, mandarin oil, or tangerine oil; however, signs of phototoxicity were observed in response to undiluted lime oil and lime oil diluted to concentrations of 15%; undiluted bitter orange oil, and undiluted lemon oil and lemon oil diluted to concentrations of 50%. In human studies, phototoxic reactions were observed in response to undiluted bitter orange oil, lemon oil (1%), sweet orange oil (1%), and undiluted and diluted expressed lime oil (30%). Many of the citrus-derived peel oils contain constituents that are photoactive agents, although those noted to be furocoumarin-free tended not to induce photosensitization.[19,30,38-41]

## Occupational Exposure

In a retrospective study (2001-2010) of professional food handlers in Denmark, 8.5% (16/188) of the patients had positive skin prick test reactions to orange peel and 7.9% (15/191) of the patients had positive skin prick test reactions to lemon peel.[42]

## SUMMARY

The 14 citrus-derived peel oils described in this report function primarily as skin conditioning agents-miscellaneous and fragrance. Botanicals such as citrus are composed of hundreds of constituents, some of which have the potential to cause toxic effects; for example, bergapten (aka 5-methoxypsoralen or 5-MOP) is a naturally-occurring, phototoxic furanocoumarin (psoralen) in citrus peel oils. Presently, CIR reviewed the information available on the potential toxicity of each of the citrus peel oil-derived ingredients as a whole, complex substance; CIR did not review the potential toxicity information on the individual constituents of which the citrus-derived ingredients are comprised. CIR requested information on the concentrations (including ranges, means, upper 95 percent confidence limits, detection limits, etc.) of individual constituents in the citrus peel oil-derived ingredients used in cosmetics, to facilitate the safety assessment of these ingredients. Such information on constituents that have been identified as of concern by the Panel in previous safety assessments, or by other recognized scientific expert review bodies, is especially important.

Citrus oils contain large amounts of monoterpene hydrocarbons; limonene is present in the greatest amount, composing 38.1-95.8% of the oils. Citrus oils also contain sesquiterpene hydrocarbons, which are responsible for the characteristic flavor of these oils.

Citrus limon (lemon) peel oil has the most reported uses in cosmetics and personal care products, with a total of 490; more than half of the uses are in leave-on skin care preparations. The range of highest maximum use concentrations for citrus limon (lemon) peel oil is 0.0001% to 0.5%, with 0.5% reported in “other” skin care preparations. Citrus aurantium dulcis (orange) peel oil (reported as citrus sinensis (sweet orange) peel oil to the VCRP) has the second greatest number of overall uses reported, with a total of 289; about half of those uses are in leave-on skin care preparations. Citrus aurantium dulcis (orange) peel oil had a highest maximum use concentration range of 0.00002% to 29%, with 29% reported in non-coloring hair conditioners.

Under the rules governing cosmetic products in the European Union, citrus-derived ingredients must have furocoumarin content below 1 mg/kg in sun-protection and bronzing products. IFRA also has issued standards for citrus oils and other furocoumarin-containing essential oils. Finished products that are applied to the skin, excluding rinse-off products like bath preparations and soaps, must not contain more than 0.0015% or 15 ppm 5-MOP. If the level of 5-MOP has not been determined, limits specified for individual oils should be observed, and when such oils are used in combination with other phototoxic ingredients, the potential additive effect should be taken into consideration and use levels should be reduced accordingly. Restrictions for furocoumarin-containing essential oils and limits on the amounts of citrus-derived oils in finished products have been recommended for bitter orange oil expressed, grapefruit oil expressed, lemon oil cold pressed, and lime oil expressed.

The European Union also set standards for limonene, stating that limonene must be included in the list of ingredients when its concentration exceeds 0.001% in leave-on products and 0.01% in rinse-off products.

The citrus-peel oils in this assessment are found in foods, and the daily exposure from food use would result in a much larger systemic dose than that resulting from use in cosmetic products. Essential oils, oleoresins (solvent-free), and natural extractives (including distillates) derived from some citrus fruits are GRAS for their intended use in foods for human and animal consumption,

The dermal $LD_{50}$ of undiluted mandarin peel oil (*Citrus reticulata*) and undiluted sweet orange oil was greater than 5 g/kg in rabbits. In undiluted lemon oil, the dermal $LD_{50}$ was greater than 10 g/kg in rabbits.

No genotoxic effects were observed in lemon oil or sweet orange oil in bacterial reverse mutation assays, mouse lymphoma cell mutation assays, and Chinese hamster chromosome aberration assays.

Tumor-promoting activity was observed in mouse skin exposed to undiluted essential oils of orange (sweet), lemon, grapefruit, or lime after pretreatment with DMBA. Related studies of 40%, 80%, or 100% orange oil following pretreatment with DMBA or urethane also reported tumor-promoting activity, although the effect was weaker in the mice induced with urethane. No tumorigenic effects were observed in mice tested with orange oil without pretreatment with DMBA or urethane. Survival rates of the mice, including controls, in these experiments were poor because of a very high incidence of renal disease

Irritation was observed in animals treated with unspecified concentrations of mandarin peel oil. In human subjects, no irritation was observed after topical exposure to lemon oil (up to 20%) or mandarin peel oil (8%).

In rabbits, lemon oil tested at 5% was not irritating and orange oil tested undiluted did not induce significant or irreversible damage to the eyes.

Mandarin peel oil (8% in petrolatum) was not sensitizing in human maximization tests. In studies of 250 dermatitic patients, less than 2.5% had positive reactions to bitter orange oil, lemon oil, or sweet orange oil tested at 2% in paraffin.

Phototoxic potential was observed for sweet orange oil and lemon oil in *in vitro* studies. No phototoxic responses were noted in some animal studies of lime oil, lemon oil, grapefruit oil, mandarin oil, or tangerine oil; however, phototoxic reactions were observed in response to undiluted lime oil and lime oil diluted to concentrations of 15%; undiluted bitter orange oil, and undiluted lemon oil and lemon oil diluted to concentrations of 50%. In human studies, phototoxic reactions were observed in response to undiluted bitter orange oil, lemon oil (1%), sweet orange oil (1%), and undiluted and diluted expressed lime oil (30%). Many of the citrus-derived peel oils contain constituents that are photoactive agents, although those noted to be furocoumarin-free tended not to induce photosensitization.

A retrospective occupational study of food handlers noted positive reactions to orange and lemon peels.

No published studies on reproductive and development toxicity of citrus-derived peel oils were discovered and no unpublished data were submitted to address these topics.

## DISCUSSION

The citrus ingredients in this assessment are found in foods, and daily exposures from the consumption of foods can be expected to yield much larger systemic exposures to these ingredients than those from the use of cosmetic products. Essential oils, oleoresins (solvent-free), and natural extracts (including distillates) derived from some citrus fruits are GRAS in foods and animal feeds. Additionally, volatile oils of limes, lemons, grapefruits, bitter oranges, oranges, and tangerines are used as flavoring agents. Consequently, the primary focus of this safety assessment is on the potential for irritation and sensitization from dermal exposures to the citrus ingredients.

Most of the reports that the Panel reviewed were not sufficiently detailed to enable determining how well the substances tested represent the cosmetic ingredients included in this safety assessment. For example, the genus and species of the plants from which the test substances were derived, the cultivation methods used to grow the plants, and the methods of extraction are not specified in many of these studies.

The Panel expressed concern about the potential for constituents in citrus-derived peel oils, including the furocoumarin 5-MOP, to cause phototoxicity. IFRA has issued standards for citrus oils and other furocoumarin-containing essential oils, and the Panel agreed that adherence to the IFRA standards for such constituents will prevent phototoxicity. According to these standards, finished products that are applied to the skin, excluding rinse-off products, must not contain more than 0.0015%, or 15 ppm, 5-MOP. An IFRA standard also has been issued for 7-methoxycoumarin; based on established maximum levels of this substance from commercially-available natural sources (like essential oils, extracts and absolutes), exposure to 7-methoxycoumarin from the use of these oils and extracts is regarded to be acceptable if the level of 7-methoxycoumarin in the finished product does not exceed 100 ppm.

Additionally, based on the findings of a rodent carcinogenicity study in which tumor promotion activity may have been caused by repeated skin irritation and resultant proliferation of DMBA-treated basal cells, the Panel concluded that citrus-derived peel oils could potentially act as tumor-promoters if formulated to reach irritant levels. Thus, these botanical ingredients must be formulated to be non-irritating.

The Panel noted that, because botanical ingredients are complex mixtures, there is concern that multiple botanical ingredients may each contribute to the final concentration of a single constituent. Therefore, when formulating products, manufacturers should avoid reaching levels in final formulation of plant constituents that may cause sensitization or other adverse effects. Specific examples of constituents that could induce adverse effects are limonene, citral, and other monoterpenes, furocoumarins (such as 5- MOP and 7-methoxycoumarin).

Finally, the Panel expressed concern about pesticide residues and heavy metals that may be present in botanical ingredients. They stressed that the cosmetics industry should continue to use current good manufacturing practices (cGMPs) to limit impurities.

## CONCLUSION

The CIR Expert Panel concluded the citrus-derived peel oils are safe for use in cosmetic products, excluding rinse-off products, do not contain more than 0.0015% (15 ppm) 5-methoxypsoralen (5-MOP), and when formulated to be non-sensitizing and non-irritating.

citrus aurantifolia (lime) peel oil*
citrus aurantium amara (bitter orange) peel oil
citrus aurantium currassuviensis peel oil*
citrus aurantium dulcis (orange) peel oil
citrus clementina peel oil*
citrus grandis (grapefruit) peel oil
citrus iyo peel oil*
citrus junos peel oil
citrus limon (lemon) peel oil
citrus medica vulgaris peel oil*
citrus nobilis (mandarin orange) peel oil
citrus reticulata (tangerine) peel oil*
citrus tachibana/reticulata peel oil*
citrus tangerina (tangerine) peel oil

**Not reported to be in current use. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in this group.*

# TABLES

**Table 1. Definitions and functions of Citrus-derived ingredients.**

| Ingredient | Definition[2] | Function[2] |
|---|---|---|
| Citrus Aurantifolia (Lime) Peel Oil | Citrus Aurantifolia (Lime) Peel Oil is the volatile oil obtained from the peel of *Citrus aurantifolia.* | Fragrance Ingredients |
| Citrus Aurantium Amara (Bitter Orange) Peel Oil<br>CAS No. 68916-04-1 | Citrus Aurantium Amara (Bitter Orange) Peel Oil is the volatile oil obtained from the peel of *Citrus aurantium amara.* | Fragrance Ingredients; Skin-Conditioning Agents - Miscellaneous |
| Citrus Aurantium Currassuviensis Peel Oil | Citrus Aurantium Currassuviensis Peel Oil is the volatile oil derived from the peel of the laraha orange, *Citrus aurantium currassuviensis.* | Fragrance Ingredients |
| Citrus Aurantium Dulcis (Orange) Peel Oil<br>CAS No. 8008-57-9 | Citrus Aurantium Dulcis (Orange) Peel Oil is the volatile oil obtained by expression from the peel of *Citrus sinensis.* | Fragrance Ingredients; Skin-Conditioning Agents - Miscellaneous |
| Citrus Clementina Peel Oil | Citrus Clementina Peel Oil is the volatile oil obtained from the peel of *Citrus clementina*. | Fragrance Ingredients; Skin-Conditioning Agents - Miscellaneous |
| Citrus Grandis (Grapefruit) Peel Oil<br>CAS No. 8016-20-4 | Citrus Grandis (Grapefruit) Peel Oil is the volatile oil obtained from the peel of the grapefruit, *Citrus grandis.* | Fragrance Ingredients; Skin-Conditioning Agents - Miscellaneous |
| Citrus Iyo Peel Oil | Citrus Iyo Peel Oil is the volatile oil obtained from the peel of *Citrus iyo.* | Skin-Conditioning Agents - Emollient |
| Citrus Junos Peel Oil | Citrus Junos Peel Oil is the volatile oil obtained from the peel of *Citrus junos.* | Cosmetic Astringents |
| Citrus Limon (Lemon) Peel Oil<br>CAS No. 8008-56-8; 8020-19-7; 84929-31-7; 85085-28-5 | Citrus Limon (Lemon) Peel Oil is the volatile oil obtained from the peel of *Citrus limon.* | Fragrance Ingredients; Skin-Conditioning Agents - Miscellaneous |
| Citrus Medica Vulgaris Peel Oil | Citrus Medica Vulgaris Peel Oil is the volatile oil obtained from the peel of *Citrus medica vulgaris.* | Fragrance Ingredients |
| Citrus Nobilis (Mandarin Orange) Peel Oil<br>CAS No. 8008-31-9; 84696-35-5 | Citrus Nobilis (Mandarin Orange) Peel Oil is the oil obtained from the peel of the mandarin orange, *Citrus nobilis.* | Fragrance Ingredients; Skin-Conditioning Agents - Miscellaneous |
| Citrus Reticulata (Tangerine) Peel Oil<br>CAS No. 8008-31-9 | Citrus Reticulata (Tangerine) Peel Oil is the volatile oil obtained from the peel of *Citrus reticulata*. | Deodorant Agents; Flavoring Agents; Fragrance Ingredients |
| Citrus Tachibana/Reticulata Peel Oil | Citrus Tachibana/Reticulata Peel Oil is the volatile oil obtained from the peel of the hybrid of *Citrus tachibana* and *Citrus reticulata.* | Skin-Conditioning Agents - Emollient |
| Citrus Tangerina (Tangerine) Peel Oil | Citrus Tangerina (Tangerine) Peel Oil is the volatile oil obtained from the peel of *Citrus tangerina*. | Fragrance Ingredients; Skin-Conditioning Agents - Miscellaneous |

**Table 2. Citrus-ingredients that potentially function solely as fragrance ingredients.**

Citrus Aurantifolia (Lime) Peel Oil
Citrus Aurantium Currassuviensis Peel Oil
Citrus Medica Vulgaris Peel Oil

**Table 3. Physical and chemical properties of Citrus-derived peel oils.**

| Property | Description | Reference |
|---|---|---|
| | ***Citrus aurantifolia* (Lime) Oil** | |
| Color | colorless to greenish yellow | 8 |
| Odor | fresh citrus, intense | 8 |
| optical rotation | +34º to +47º | 8 |
| Solubility | insoluble in water, soluble in ethanol and propylene glycol | 8 |
| refractive index | 1.4477-1.4745 | 8 |
| specific gravity | 0.855-0.863 | 8 |
| | ***Citrus aurantifolia* or *Citrus latifolia* (Lime) Oil (Distilled)** | |
| Color | colorless to greenish yellow | 1 |
| Odor | mild citrus, floral | 1 |
| optical rotation/angular rotation | +34º to +47º | 1 |
| Solubility | soluble in most fixed oils and mineral oil; insoluble in glycerin and propylene glycol | 1 |
| solubility in alcohol | 1 ml sample dissolves in 5 ml of 90% alcohol | 1 |
| Aldehydes | between 0.5% and 2.5% of aldehydes, calculated as citral | 1 |
| refractive index | 1.474 – 1.477 at 20 ºC | 1 |
| specific gravity | 0.855-0.863 | 1 |
| | ***Citrus aurantifolia* (Lime) Oil (Cold-Pressed)** | |
| Color | yellow to brown green to green liquid | 1 |
| Odor | fresh lime peel | 1 |
| optical rotation/angular rotation | Mexican type: +35º to +41º; Tahitian type: +38º to +53º | 1 |
| Solubility | soluble in most fixed oils and mineral oil; insoluble in glycerin and propylene glycol | 1 |
| Aldehydes | Mexican type: no less than 4.5% and no more than 8.5% of aldehydes, calculated as citral; Tahitian type: no less than 3.2% and no more than 7.5% of aldehydes, calculated as citral | 1 |
| refractive index | Mexican type: 1.482-1.486; Tahitian type: 1.476-1.486 | 1 |
| residue of evaporation | Mexican type: 10.0% to 14.5%; Tahitian type: 5.0% to 12.0% | 1 |
| specific gravity | Mexican type: 0.872-0.881; Tahitian type: 0.858-0.876 | 1 |
| UV absorbance | max. at 315 nm; Mexican type: no less than 0.45; Tahitian type: no less than 0.24 | 1 |
| | ***Citrus aurantium* (Bitter Orange) Oil (Cold-Pressed)** | |
| Color | pale yellow or yellow-brown liquid | 1 |
| Odor | characteristic aromatic odor of the Seville orange | 1 |
| optical rotation/angular rotation | +88º to +98º | 1 |
| Solubility | miscible with absolute alcohol and with an equal volume of glacial acetic acid; soluble in fixed oils and mineral oil; slightly soluble in propylene glycol; relatively insoluble in glycerin | 1 |
| Aldehydes | no less than 0.5% and no more than 1.0% of aldehydes, calculated as decyl aldehyde | 1 |
| refractive index | 1.472-1.476 at 20º C | 1 |
| residue of evaporation | 2.0-5.0% | 1 |
| specific gravity | 0.845-0.851 | 1 |
| | ***Citrus clementina*** | |
| color | orange to brownish liquid | 43 |
| odor | characteristic | 43 |
| optical rotation | +89 - +97º (15ºC) | 43 |
| solubility | insoluble in water; soluble in ethyl alcohol | 43 |
| refractive index | 1.5640 – 1.4820 (20ºC) | 43 |
| specific gravity | 0.840 – 0.875 (15ºC) | 43 |
| | ***Citrus limon* (Lemon) Oil** | |
| Color | pale to deep yellow or greenish yellow | 8 |
| Odor | fresh citrus, intense | 8 |
| optical rotation | +57 to +65.6 | 8 |
| Solubility | insoluble in water, soluble in ethanol and propylene glycol | 8 |
| refractive index | 1.474-1.467 | 8 |
| specific gravity | 0.849-0.855 | 8 |
| | ***Citrus limon* (Lemon) Oil (Distilled)** | |
| Color | colorless to pale yellow liquid | 1 |
| Odor | fresh lemon peel | 1 |
| optical rotation/angular rotation | +55º to +75º | 1 |
| Solubility | soluble in most fixed oil, mineral oil, and alcohol (with haze); insoluble in glycerin and propylene glycol | 1 |
| solubility in alcohol | 1 ml sample dissolves in 5 ml of 90% alcohol | 1 |
| Aldehydes | between 1.0% and 3.5% of aldehydes, calculated as citral | 1 |
| refractive index | 1.470 – 1.475 at 20 ºC | 1 |
| specific gravity | 0.842-0.856 | 1 |
| UV absorbance | max. at 315 nm, no less than 0.01 | 1 |
| | ***Citrus limon* (Lemon) Oil (Cold-Pressed)** | |
| Color | pale to deep yellow or green-yellow | 1 |
| Odor | fresh lemon peel | 1 |
| optical rotation/angular rotation | California and Italian types: +57º to +65.6º; Desert type: +67º to +78º | 1 |

**Table 3. Physical and chemical properties of Citrus-derived peel oils.**

| Property | Description | Reference |
|---|---|---|
| Solubility | miscible with dehydrated alcohol and glacial acetic acid | [1] |
| solubility in alcohol | 1 ml of sample dissolves in 3 ml of 95% alcohol, slight haze possible | [1] |
| Aldehydes | Desert type: no less than 1.7% of aldehydes, calculated as citral; California type: no less than 2.2% and no more than 3.8% of aldehydes, calculated as citral; Italian type: no less than 3.0% and no more than 5.5% of aldehydes, calculated as citral | [1] |
| refractive index | 1.473-1.476 at 20 ºC | [1] |
| residue of evaporation | between 5.0% to 14.5% | [1] |
| specific gravity | Desert type: 0.846-0.851; California and Italian types: 0.849-0.855 | [1] |
| UV absorbance | max. at 315 nm; Desert and California types: no less than 0.2; Italian type: no less than 0.49 | [1] |
| ***Citrus nobilis* or *Citrus reticulata* (Tangerine) Oil (Cold-Pressed)** | | |
| Color | red-orange to brown-orange | [1] |
| Odor | pleasant, orange | [1] |
| optical rotation/angular rotation | +88º to +96º | [1] |
| Solubility | soluble in most fixed oils and mineral oil; slightly soluble in propylene glycol; relatively insoluble in glycerin | [1] |
| Aldehydes | 0.8% to 1.9% of aldehydes, calculated as decyl aldehyde | [1] |
| refractive index | 1.473-1.476 at 20 ºC | [1] |
| residue of evaporation | 2.3% - 5.8% | [1] |
| specific gravity | 0.844-0.854 | [1] |
| **Citrus Reticulata Peel Oil** | | |
| physical state and appearance | clear, mobile, dark-orange to reddish-orange or brownish-orange liquid | [10] |
| Odor | orange-like | [10] |
| optical rotation | +63° to +78° | [10] |
| refractive index | 1.4730-1.4770 (20°C) | [10] |
| specific gravity | 0.847-0.853 (25/25°C) | [10] |
| ***Citrus sinensis* (Orange) Oil (Distilled)** | | |
| Color | colorless to pale yellow liquid | [1] |
| Odor | mild citrus floral | [1] |
| optical rotation/angular rotation | +94º to +99º | [1] |
| Solubility | soluble in most fixed oil, mineral oil, and alcohol (with haze); insoluble in glycerin and propylene glycol | [1] |
| solubility in alcohol | 1 ml sample dissolves in 5 ml of 90% alcohol | [1] |
| refractive index | 1.471 – 1.474 at 20 ºC | [1] |
| specific gravity | 0.840-0.844 | [1] |
| UV absorbance | max. at 330 nm, no less than 0.01 | [1] |
| ***Citrus sinensis* (Orange) Oil (Cold-Pressed)** | | |
| Color | intensely yellow, orange, or deep orange liquid | [1] |
| Odor | characteristic of fresh, sweet orange peel | [1] |
| optical rotation/angular rotation | +94º to +99º | [1] |
| Solubility | miscible with dehydrated alcohol and carbon disulfide; soluble in glacial acetic acid | [1] |
| Aldehydes | no less than 1.2% and no more than 2.5% of aldehydes, calculated as decyl aldehyde | [1] |
| refractive index | 1.472-1.474 at 20 ºC | [1] |
| specific gravity | 0.842-0.846 | [1] |
| UV absorbance | max. at 330 nm; California type: no less than 0.130; Florida type: no less than 0.240 | [1] |

**Table 4. Chemical composition (%) of citrus peel oils***

| | ***C. aurantifolia* (lime) peel oil** | | ***C. aurantium amara* (bitter orange) essential oil** | | | | ***C. aurantium dulcis* (orange) essential oil** | | ***C. clementina* peel essential oil** | | ***C. grandis* (grape-fruit) peel essential oil** | | ***C. iyo* peel essential oil** | | ***C. junos* peel essential oil** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **cold-pressed**[13] | | **cold-pressed**[14] | | **hydrodistilled**[18,20] | | **cold-pressed**[14] | | **hydrodistillation**[17] | | **cold-pressed**[6] | | **lyophilization/ vacuum distillation**[9] | | **cold-pressed**[7] **or not specified**[16] | |
| | **Min** | **Max** | **Min** | **Max** | **Min** | **Max** | **Min** | **Max** | **Min** | **Max** | **Min** | **Max** | **Min** | **Max** | **Min** | **Max** |
| **Essential Oil Content (%)** | | | | | **0.12**[18] **- 1.67**[20] | | | | | | **0.12**[6] | | **1.5**[9] | | | |
| **Constituent** | | | | | | | | | | | | | | | | |
| limonene | 39.9 | **94.4** | | | 67.9 | **94.67** | | | 93.77 | **96.19** | | **94.8** | 79 | **89** | **63.1** | **77.44** |
| (*E*)-α-bergamotene | | | | | | | | | | | | | | | trace | |
| (*E*)-*β*-farnesene | trace | 0.1 | | | | | | | | | | | | | trace | |
| (*E*)-*β*-ocimene | trace | 0.5 | | | 0.02 | 0.5 | | | | | | | | | ND | trace |
| (*E*)-decenal | | | | | | | | | | | | | | | ND | trace |
| (*E*)-carvone | | | | | | | | | | | | 0.005-0.05 | | | | |
| (*E*)-caryophyllene | 0.1 | 0.5 | | | | | | | | | | | | | | |
| (*E*)-caryophyllene oxide | | | | | | | | | | | | trace | | | | |
| (*E*)-linalool oxide | | | | | | | | | | | | | | | ND | trace |
| (*E*)-nerolidol | | | | | | | | | | | | | | | trace | |
| (*E*)-p-mentha-2,8-dienol | | | | | | | | | | | | 0.1 | | | | |
| (*E*)-p-2,8-menthadien-1-ol | | | | | | | | | | | | trace | | | | |
| (*E*,*E*)-α-farnesene | 0.3 | 1.9 | | | | | | | | | | 0.1 | | | | |
| (*E*,*E*)-farnesyl acetate | | | | | | | | | | | | | | | ND | trace |
| (*E*,*Z*)-2,6-nonadien-1-ol | | | | | | | | | | | ND | | | | | |
| (Z)-*β*-ocimene | trace | 0.7 | | | | | | | | | | | | | trace | |
| *Z*-carveol | | | | | | | | | | | | 0.005-0.05 | | | | |
| (*Z*)-carvone | | | | | | | | | | | | 0.1 | | | | |
| (*Z*)-β-farnesene | | | | | | | | | | | | | | | 0.9 | 1.3 |
| (*Z*)-limonene oxide | | | | | | | | | | | | | | | ND | trace |
| (*Z*)-piperitol | | | | | | | | | | | | 0.005-0.05 | | | | |
| 1-octanol | | | | | | | | | | | | | | | | |
| 1,3,8-*p*-menthatriene | | | | | | | | | | | | | | | trace | |
| 1,8-cineole | 0.5 | 0.6 | | | trace | 14.72 | | | | | | | | | | |
| 2Z.6E-farnesol | | | | | trace | | | | | | | | | | | |
| 3-carene | trace | 0.3 | | | | | | | | | | | | | | |
| 3,3’,4’,5,6,7-hepta-methoxyflavone | | | X | | | | X | | | | | | | | | |
| 3,3’,4’,5,6,7-hexameth-oxyflavone | | | | | | | X | | | | | | | | | |
| 3’,4’,5,6,7,8-hexameth-oxyflavone | | | X | | | | | | | | | | | | | |
| 3’,4’,5,6,7-pentameth-oxyflavone | | | | | | | X | | | | | | | | | |
| 4’,5,6,7-tetramethoxy-flavone | | | X | | | | X | | | | | | | | | |
| 4’,5,6,7,8-pentamethoxy-flavone | | | X | | | | X | | | | | | | | | |
| 5-methoxypsoralen | | | X | | | | | | | | | | | | | |

**Table 4. Chemical composition (%) of citrus peel oils***

| | *C. aurantifolia* (lime) peel oil | | *C. aurantium amara* (bitter orange) essential oil | | | | *C. aurantium dulcis* (orange) essential oil | | *C. clementina* peel essential oil | | *C. grandis* (grapefruit) peel essential oil | | *C. iyo* peel essential oil | | *C. junos* peel essential oil | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | cold-pressed[13] | | cold-pressed[14] | | hydrodistilled[18,20] | | cold-pressed[14] | | hydrodistillation[17] | | cold-pressed[6] | | lyophilization/ vacuum distillation[9] | | cold-pressed[7] or not specified[16] | |
| | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max |
| (5-(6',7'-dihydroxy-geranyloxy)psolaren) | | | X | | | | | | | | | | | | | |
| (5-(6',7'-epoxy-geranyl-oxy)psolaren) | | | X | | | | | | | | | | | | | |
| 5,7-dimethoxycoumarin | | | X | | | | | | | | | | | | | |
| 6-methylhept-5-en-2-one | | trace | | | | | | | | | | | | | | |
| 7-methoxy-8(2',3'-dihy-droxy)isopentyloxy-coumarin | | | X | | | | | | | | | | | | | |
| 7-methyoxy-8(2',3'-epoxy)isopentenylcou-marin | | | X | | | | | | | | | | | | | |
| 7-methyoxy-8-iso-pentenylcoumarin | | | X | | | | | | | | | | | | | |
| 7-methoxy-8-(2'-one-iso-pentyl)coumarin) | | | X | | | | | | | | | | | | | |
| 30 carvacrol | | | | | trace | 0.69 | | | | | | | | | | |
| *allo*-ocimene | | trace | | | | | | | | | | | | | | |
| aromadendrene | | | | | | | | | | | | | | | ND | trace |
| bicycloelemene | | | | | | | | | | | | | | | trace | |
| bicyclogermacrene | trace | 0.4 | | | | | | | | | | | | | 0.7 | 2.0 |
| borneol | | | | | 0.08 | 0.16 | | | | | | | | | | |
| bornyl acetate | trace | trace | | | trace | | | | | | | | | | | |
| camphene | trace | 0.2 | | | trace | 0.24 | | | | | | | | | trace | |
| camphor | | | | | 0.17 | 6.37 | | | | | | | | | | |
| caryophyllene | | | | | | | | | | | | | | | ND | trace |
| caryophyllene oxide | trace | 0.1 | | | 0.08 | 0.21 | | | | | | trace | | | | |
| cedrol | | | | | | | | | | | trace | | | | | |
| *cis*-dihydrocarvone | | | | | | | 0.03 | 0.28 | | | | | | | | |
| *cis*-limonene-1,2-oxide | trace | 0.1 | | | | | | | | | | | | | | |
| *cis*-linalool oxide THF | | trace | | | trace | 3.4 | | | | | | | | | | |
| *cis*-β-ocimene | | | | | | 0.30 | | | | | | | | | | |
| *cis*-sabinene hydrate | | | | | trace | 0.18 | | | | | | | | | trace | 0.1 |
| citronellal | trace | 1.6 | | | | | | | 0.03 | 0.08 | | 0.1 | | | | |
| citronellol | trace | 0.6 | | | | | trace | 0.07 | | | | | | | ND | trace |
| citronellyl acetate | 0.1 | 0.2 | | | | | | | | | | | | | | |
| cumin aldehyde | | | | | | | | | | | | 0.005-0.05 | | | | |
| decanal | trace | 0.3 | | | | 0.16 | | | | | | 0.3 | | | trace | |
| dihydrocarveol | | | | | | | | | | | trace | | | | | |
| dodecanol | | trace | | | | | | | | | | 0.1 | | | ND | trace |
| elemol | | | | | | | | | | | trace | | | | trace | |
| eugenol | | | | | | | | | | | | | | | ND | trace |
| geranial | 0.5 | 6.1 | | | | | | | | | | 0.1 | | | | |
| geraniol | trace | 0.3 | | | | | | | | | | | | | | |
| geranyl acetate | 0.1 | 2.4 | | | trace | 0.11 | | | | | | | | | | |

**Table 4. Chemical composition (%) of citrus peel oils***

| | *C. aurantifolia* (lime) peel oil | | *C. aurantium amara* (bitter orange) essential oil | | | | *C. aurantium dulcis* (orange) essential oil | | *C. clementina* peel essential oil | | *C. grandis* (grape-fruit) peel essential oil | | *C. iyo* peel essential oil | | *C. junos* peel essential oil | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | cold-pressed[13] | | cold-pressed[14] | | hydrodistilled[18,20] | | cold-pressed[14] | | hydrodistillation[17] | | cold-pressed[6] | | lyophilization/ vacuum distillation[9] | | cold-pressed[7] or not specified[16] | |
| | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max |
| geranyl propionate | | | | | | | | | | | trace | | | | | |
| germacrene B | | | | | | | | | | | | | | | 0.1 | 0.2 |
| germacrene D | trace | 1.0 | | | 0.04 | 0.25 | | | | | | | | | 0.3 | 0.4 |
| germacrene D-4-ol | | | | | | | | | | | | | | | 0.3 | 0.4 |
| globulol | | 0.1 | | | | | | | | | | | | | | |
| guaiene | | | | | | | | | | | | | | | ND | trace |
| heptyl acetate | | | | | | | | | | | | 0.3 | | | | |
| isothyujol | | | | | | | | | | | | 0.005-0.05 | | | | |
| limonen-10-ol | | | | | | | | | | | trace | | | | | |
| linalool | 0.1 | 1.4 | | | trace | 0.76 | | | 0.03 | 0.08 | | 0.1 | 1.3 | 7.3 | 1.6 | 2.8 |
| linalool oxide | | | | | | | | | | | | | | | | |
| linalyl acetate | trace | trace | | | 0.01 | 0.18 | | | | | | 0.1 | | | | |
| methyl-N-methylanthranilate | | | | | | | | | | | | 0.005-0.05 | | | | |
| myrcene | 0.8 | 2.0 | | | 0.07 | 2.0 | | | 0.73 | 0.78 | | | X | | 0.4 | 3.2 |
| neral | trace | 3.1 | | | | | | | | | trace | | | | | |
| nerol | trace | 0.1 | | | 0.03 | 0.12 | | | | | trace | | | | ND | trace |
| nerolidyl acetate | | | | | | | | | | | trace | | | | | |
| neryl acetate | trace | 2.0 | | | | 0.10 | | | | | | | | | | |
| nonanal | trace | 0.1 | | | trace | 0.18 | | | | | | 0.005-0.05 | | | trace | |
| nonanoic acid | | | | | | | | | | | | trace | | | | |
| nootkatone | | | | | | | | | | | | 0.1. | | | | |
| octanal | trace | 0.1 | | | 0.02 | 1.59 | | | | | | 0.4 | | | trace | |
| octyl acetate | trace | trace | | | | | | | | | | | | | | |
| *p*-cymene | trace | 5.3 | | | trace | 0.09 | | | | | | | | | 0.4 | 0.6 |
| *p*-cymenene | | 0.1 | | | | | | | | | | | | | | |
| *p*-mentha-1-en-9-ol | | | | | | | | | | | trace | | | | | |
| perillaldehyde | | | | | | | | | | | trace | | | | trace | |
| perillyl alcohol | | | | | | | | | | | | | | | trace | |
| perillyl aldehyde | | | | | | | | | | | | 0.005-0.05 | | | | |
| piperitenone | | trace | | | | | | | | | | | | | trace | |
| psuedolimonene | | | | | | | | | | | | | | | trace | |
| sabina ketone | | | | | | | | | | | | 0.1 | | | | |
| sabinene | 0.1 | 19.6 | | | trace | 0.200.18 | | | | | | 0.4 | | | 0.4 | 0.5 |
| santal-10-en-2-ol | trace | trace | | | | | | | | | | | | | | |
| spathulenol | | | | | trace | 0.21 | | | | | | | | | ND | trace |
| terpinen-4-ol | trace | 1.7 | | | 0.01 | 0.54 | | | | | | | | | trace | 0.1 |
| terpinolene | trace | 1.1 | | | 0.02 | 0.22 | | | | | ND | | | | 0.6 | 0.7 |
| terpinyl acetate | trace | trace | | | | | | | | | | | | | | |
| tetradecane | | | | | | | | | | | | | | | ND | trace |
| tetradecenal | | | | | | | | | | | trace | | | | | |
| thymol | | | | | | | | | | | | | | | 0.2 | 0.3 |
| thymyl methyl oxide | 1.2 | 1.6 | | | | | | | | | | | | | | |
| *trans*-α-bergamotene | 0.2 | 1.6 | | | | | | | | | | | | | | |

**Table 4. Chemical composition (%) of citrus peel oils***

| | *C. aurantifolia* (lime) peel oil | | *C. aurantium amara* (bitter orange) essential oil | | | | *C. aurantium dulcis* (orange) essential oil | | *C. clementina* peel essential oil | | *C. grandis* (grape-fruit) peel essential oil | | *C. iyo* peel essential oil | | *C. junos* peel essential oil | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | cold-pressed[13] | | cold-pressed[14] | | hydrodistilled[18,20] | | cold-pressed[14] | | hydrodistillation[17] | | cold-pressed[6] | | lyophilization/ vacuum distillation[9] | | cold-pressed[7] or not specified[16] | |
| | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max |
| *trans*-limonene-1,2-oxide | trace | 0.2 | | | | | | | | | | | | | | |
| *trans*-sabinene hydrate | trace | 0.1 | | | | | | | | | | | | | trace | 0.1 |
| tricyclen | | | | | 0 | 0.75 | | | | | | | | | | |
| undecanal | trace | 0.1 | | | | | | | | | | | | | | |
| valencene | | | | | 0.04 | 0.2 | | | | | | | | | | |
| (-)-α-copaene | | | | | | | | | | | | | | | trace | 0.1 |
| α-cadinene | | | | | | | | | | | | | | | | 0.1 |
| α-cadinol | | | | | | | | | | | | | | | ND | trace |
| α-cedrene | | | | | | | | | | | | trace | | | | |
| α-copaene | | | | | | | | | | | | 0.005-0.05 | | | | |
| α-cubebene | | | | | | | | | | | | 0.1 | | | trace | |
| α-farnesene | | | | | | | | | | | | | | | ND | trace |
| α-humulene | trace | 0.2 | | | 0.03 | 0.13 | | | | | | | | | | 0.1 |
| α-muurolene | | | | | | | | | | | | | | | trace | |
| α-p-dimethyl styrene | | | | | | | | | | | | | | | trace | |
| α-phellandrene | trace | 0.1 | | | | | | | | | | | | | 0.7 | 0.9 |
| α-pinene | 0.2 | 2.7 | | | 0.03 | 0.53 | | | 0.15 | 0.31 | | 0.5 | | | 2.0 | 2.7 |
| α-sinensal | | | | | | | | | | | | | | | | |
| α-terpinene | trace | 0.4 | | | 0.91 | 1.66 | | | | | | 1.8 | X | | | 0.3 |
| α-terpineol | trace | 4.7 | | | 0.07 | 0.35 | | | 0.04 | 0.09 | | 0.1 | | | 0.2 | 0.3 |
| α-terpinyl acetate | | | | | trace | 1.4 | | | | | | | | | | |
| α-thujene | trace | 0.7 | | | 0.14 | 0.44 | | | | | ND | | | | | |
| α-thujone | | | | | trace | 0.35 | | | | | | | | | | |
| α-ylangene | | | | | | | | | | | | | | | ND | trace |
| β-bisabolene | 0.3 | 2.4 | | | | | | | | | | | | | | |
| β-caryophyllene | | | | | | | | | | | | 0.1 | | | | 0.3 |
| β-cubebene | | | | | | | | | | | | | | | trace | 0.1 |
| β-eleme | trace | 0.8 | | | | | | | | | | | | | | 0.1 |
| β-eudesmol | | | | | | | | | | | | | | | trace | |
| β-ionone | | | | | | | | | | | | | | | ND | trace |
| β-phellandrene | 0.2 | 0.8 | | | | | | | | | | | | | 2.16 | 5.4 |
| β-pinene | trace | 19.2 | | | 0.07 | 0.62 | | | 0.06 | 0.36 | | 0.005-0.05 | | | 0.9 | 1.1 |
| β-sesquiphellandrene | | | | | | | | | | | | | | | 0.1 | |
| β-sinensal | trace | 0.1 | | | | | | | | | trace | | | | | |
| γ-terpinene | trace | 21.5 | | | 0.14 | 0.31 | | | | | | 0.005-0.05 | | | 11.4 | 12.3 |
| δ-carene | | | | | | | | | | | | | | | ND | trace |
| δ-elemene | | | | | | | | | | | trace | | | | 0.1 | 0.2 |
| δ-3-carene | | | | | | 0.17 | | | | | | | | | | |
| $\Delta^3$-carene | | | | | trace | 0.01 | | | | | | | | | | |

**Table 4. Chemical composition (%) of citrus peel oils***

| | ***C. limon* (lemon) peel essential oils** | | | | ***C. nobilis* (mandarin orange) essential oil** | | ***C. sinensis* (orange) peel oil** | | | | | | ***C. reticulata* (tangerine) peel oil** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **cold-pressed**[12,13] | | **hydrodistilled**[18] | | **cold-pressed**[14] | | **cold-pressed**[11,12,15,16] | | **hydrodistilled**[44] | | **steam-distilled**[22] | | **hydrodistilled**[21,44] | | | |
| | **Min** | **Max** | **Min** | **Max** | **Min** | **Max** | **Min** | **Max** | **Min** | **Max** | **Min** | **Max** | **Min** | **Max** | | |
| **Essential Oil Content (%)** | **1.12**[12] | | **1.3**[18] | | | | **0.98-1.21**[12] | | **0.24-1.07**[44] | | **2.49**[22] | | **0.30-0.50**[44] | | | |
| **Constituent** | | | | | | | | | | | | | | | | |
| limonene | 38.1 | **95.8** | 37.631 | **69.71** | | | 61.08 | **96.57** | 66.8 | **80.9** | | **77.49** | 64.1 | **92.4** | | |
| (*E*)-*β*-farnesene | trace | 0.2 | | | | | | | | | | | | | | |
| (*E*)-*β*-ocimene | trace | 0.4 | | | | | | 0.1 | | | | | | | | |
| (*E*)-caryophyllene | trace | 0.7 | | | | | | | | | | | | | | |
| (*E*)-nerolidol | trace | trace | | | | | | | | | | | | | | |
| (Z)-*β*-ocimene | trace | 0.3 | trace | 0.5 | | | | | | 0.34 | | | | 0.28 | | |
| *Z*-carveol | | | | | | | | | 0.68 | 4.53 | | | | 1.29 | | |
| 1-octanol | | | | | | | | | | | | | 0.55 | 0.95 | | |
| 1,3,8-*p*-menthatriene | | | | | | | | | 1.21 | 1.69 | | | | | | |
| 1,8-cineole | | | trace | 0.82 | | | | | | | | | | | | |
| 2Z.6E-farnesol | | | trace | | | | | | | | | | | | | |
| 3-carene | trace | trace | | | | | | | | | | | | | | |
| 3-furanacetic acid | | | | | | | | | | 3.0 | | | | | | |
| 3,3’,4’,5,6,7-hepta-methoxyflavone | | | | | X | | | | | | | | | | | |
| 3’,4’,5,6,7,8-hexameth-oxyflavone | | | | | X | | | | | | | | | | | |
| 4-carene | | | | | | | | | 0.61 | 0.85 | | | | | | |
| 4-carvon menthenol | | | | | | | | | | | | | | 0.95 | | |
| 4-terpineol | | | | | | | | | | | | 0.43 | | | | |
| 4-vinyl guaiacol | | | | | | | | | 1.21 | 1.27 | | | 0.87 | 2.32 | | |
| 4’,5,6,7-tetramethoxy-flavone | | | | | X | | | | | | | | | | | |
| 4’,5,6,7,8-pentamethoxy-flavone | | | | | X | | | | | | | | | | | |
| 6-methylhept-5-en-2-one | trace | trace | | | | | | | | | | | | | | |
| 30 cavacrol | | | trace | 0.69 | | | | | | | | | | | | |
| allyl isovalerate | | | | | | | | | | | | | | 0.36 | | |
| benzaldehyde | | 0.11 | | | | | | | | | | | | | | |
| bicyclogermacrene | | | | | | | | trace | | | | | | | | |
| borneol | | 0.16 | 0.33 | 2.54 | | | | 7.63 | | | | | | | | |
| bornyl acetate | trace | trace | trace | 0.01 | | | | | | | | | | | | |
| camphene | trace | 0.13 | trace | 0.24 | | | | 0.32 | | | | | | | | |
| camphor | | | trace | 0.58 | | | | | | | | | | | | |
| capraldehyde | | | | | | | 0.35 | 5.62 | | | | | | | | |
| caprinaldehyde | | 0.26 | | | | | | 2.10 | | | | | | | | |
| caryophyllene oxide | trace | 0.3 | trace | | | | | | | | | | | | | |
| carvone | | | | | | | | | | | | 0.17 | 0.47 | 0.62 | | |
| d-carvone | | | | | | | | | | 0.39 | | | | | | |
| cephrol | | | | | | | | | | | | 0.15 | | | | |
| *cis*-carveol | | | | | | | | | | | | 0.22 | | | | |
| *cis*-dihydrocarvone | | | trace | 0.29 | | | | | | | | | | | | |
| *cis*-limonene-1,2-oxide | trace | 0.5 | | | | | | | | | | | | | | |
| *cis*-linalool oxide THF | | | 0 | 0.49 | | | | | | | | | | | | |
| *cis*-sabinene hydrate | | | trace | 0.05 | | | | | | | | | | | | |

**Table 4. Chemical composition (%) of citrus peel oils***

| | *C. limon* (lemon) peel essential oils | | | | *C. nobilis* (mandarin orange) essential oil | | *C. sinensis* (orange) peel oil | | | | | | *C. reticulata* (tangerine) peel oil | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | cold-pressed[12,13] | | hydrodistilled[18] | | cold-pressed[14] | | cold-pressed[11,12,15,16] | | hydrodistilled[44] | | steam-distilled[22] | | hydrodistilled[21,44] | | | |
| | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | | |
| *cis*-β-farnesene | | | | | | | | | | | | | | 0.1 | | |
| *cis*-β-ocimene | | | | | | | | 0.26 | | | | | | | | |
| citral | | 0.27 | | | | | 1.74 | 7.74 | | | | | | | | |
| citranelyl acetate | | | | | | | 0.01 | 0.22 | | | | | | | | |
| citronellal | trace | 0.4 | | | | | 0.06 | 0.2 | | | | | 0.45 | 0.78 | | |
| citronellol | trace | 0.15 | trace | 0.04 | | | | 4.18 | 0.71 | 1.6 | | | 0.8 | 1.2 | | |
| citronellyl acetate | trace | 0.3 | | | | | | | | | | 0.12 | | | | |
| copaene | | | | | | | | | | | | | | 0.89 | | |
| decanal | trace | trace | | | | | | 0.21 | 0.71 | 1.02 | | 0.11 | 2.33 | 7.71 | | |
| decanol | | | | | | | | 0.35 | | | | | | | | |
| dehydrolinalool | | | | | | | | | | | | 0.40 | | | | |
| dodecanol | | | | | | | | 0.03 | | | | | 0.42 | 0.65 | | |
| elemol | | | | | | | | | | | | | 0.35 | 0.89 | | |
| eugenol | | | | | | | | | | 0.53 | | | | | | |
| farnesol | | | | | | | | | | | | | | 1.14 | | |
| geranial | 0.1 | 2.9 | | | | | trace | 0.11 | | | | | | | | |
| geraniol | trace | 0.2 | | | | | | | | | | | | | | |
| geranyl acetate | trace | 3.2 | trace | 0.56 | | | | | | | | | | | | |
| germacarene | | | | | | | | | | | | | 0.30 | 1.07 | | |
| germacrene D | trace | 0.8 | 0.019 | 1.35 | | | | 0.08 | | | | 0.27 | | | | |
| n-heptane | | | | | | | | | | | | | | 0.4 | | |
| hexadecane | | | | | | | | 0.2 | | | | | | | | |
| isophorone | | | | | | | | | 1.09 | 2.92 | | | | | | |
| isopropyl cresol | | | | | | | | | | | | | | 1.36 | | |
| lemonol | | | | | | | | | | | | 0.28 | | | | |
| limonene oxide | | | | | | | | | | 0.76 | | | | | | |
| linalool | 0.1 | 25.1 | 0 | 1.59 | | | 0.12 | 2.32 | 1.52 | 2.1 | | 0.22 | 0.7 | 2.56 | | |
| linalool oxide | | | | | | | | | 0.48 | 1.12 | | | | 0.6 | | |
| linalyl acetate | trace | 31.2 | trace | 2.61 | | | trace | | | | | 0.28 | | 0.4 | | |
| longipinene | | | | | | | | | | | | | | 0.4 | | |
| menthadien-1-ol | | | | | | | | | | | | | | 0.42 | | |
| myrcene | 0.7 | 10.16 | trace | 1.54 | | | | 2.5 | | | | 6.27 | | | | |
| neral | trace | 1.5 | | | | | | 0.06 | | | | | | | | |
| nerol | trace | 0.1 | trace | 0.24 | | | | | | | | | | | | |
| neryl acetate | 0.1 | 3.9 | | | | | | 0.04 | | | | | | | | |
| nikkol | | | | | | | | | 5.7 | | | | | | | |
| nonanal | trace | 0.3 | trace | | | | | 0.08 | | | | | | | | |
| n-nonane | | | | | | | | 0.2 | | | | | | | | |
| nootkatone | | | | | | | | 0.01 | | | | | 0.30 | 3.95 | | |
| octanal | trace | 0.1 | 0.02 | 1.59 | | | 0.34 | 0.8 | | | | | 1.06 | 1.15 | | |
| octyl acetate | trace | 0.1 | | | | | | | | | | | | | | |
| *p*-cymene | 0.1 | 7.8 | 0.23 | 9.84 | | | | | | | | | | | | |
| *p*-cymenene | trace | 0.3 | | | | | | | | | | | | | | |
| pentadecane | | | | | | | | 0.2 | | | | | | | | |
| perillaldehyde | | | | | | | | 0.03 | | | | | 1.64 | 1.65 | | |
| piperitenone | | | | | | | | | | 0.42 | | | | | | |
| sabinene | trace | 6.3 | 3.8 | 6.48 | | | 0.21 | 0.5 | 0.37 | 0.62 | | 1.29 | | 0.49 | | |

**Table 4. Chemical composition (%) of citrus peel oils***

| | ***C. limon* (lemon) peel essential oils** | | | | ***C. nobilis* (mandarin orange) essential oil** | | ***C. sinensis* (orange) peel oil** | | | | | | ***C. reticulata* (tangerine) peel oil** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **cold-pressed**[12,13] | | **hydrodistilled**[18] | | **cold-pressed**[14] | | **cold-pressed**[11,12,15,16] | | **hydrodistilled**[44] | | **steam-distilled**[22] | | **hydrodistilled**[21,44] | | | |
| | **Min** | **Max** | **Min** | **Max** | **Min** | **Max** | **Min** | **Max** | **Min** | **Max** | **Min** | **Max** | **Min** | **Max** | | |
| spathulenol | trace | 0.1 | trace | | | | | | | | | | | | | |
| terpineol-4 | trace | 0.1 | | | | | | | | | | | | | | |
| terpinen-4-ol | | | trace | 1.28 | | | | | | | | | | 0.1 | | |
| terpinolene | trace | 0.8 | 0.02 | 0.22 | | | | 0.08 | | | | | | | | |
| terpinyl acetate | | 0.5 | trace | 0.15 | | | | | | | | | | | | |
| tetradecane | | | | | | | | 0.2 | | | | | | | | |
| thymol | trace | 1.1 | | | | | | trace | | | | | | | | |
| *trans*-α-bergamotene | 0.2 | 1.3 | | | | | | | | | | | | | | |
| *trans*-carveol | | | | | | | | | | | | 0.44 | | | | |
| *trans*-limonene oxide | | | | | | | | 0.01 | | | | 0.20 | | | | |
| *trans*-limonene-1,2-oxide | trace | 0.6 | | | | | | | | | | | | | | |
| *trans*-linalool oxide THF | trace | trace | | | | | | | | | | | | | | |
| *trans*-p-1,8-dienol | | | | | | | | | | | | | | 0.52 | | |
| *trans*-*p*-menth-2-en-1-ol | trace | 0.1 | | | | | | | | | | | | | | |
| *trans*-p-2,8-menthadien-1-ol | | | | | | | | | 0.79 | 1.07 | | | | | | |
| *trans*-sabinene hydrate | trace | 0.1 | | | | | | | | | | | | | | |
| tricyclen | | | 0.02 | 0.16 | | | | | | | | | | | | |
| valencene | | | trace | 0.37 | | | | 0.05 | 0.41 | 1.2 | | | | | | |
| α-bisabolene | | 0.2 | | | | | | | | | | | | | | |
| α-cadinol | | | | | | | | | | | | | | 0.46 | | |
| α-copaene | | | | | | | | 0.04 | | | | | | | | |
| α-cubebene | | | | | | | | | | 0.7 | | | | 0.48 | | |
| α-farnesene | | | | | | | | | | | | | 0.36 | 0.48 | | |
| α-humulene | trace | 0.2 | trace | 0.04 | | | | | | | | | | | | |
| α-phellandrene | trace | 0.6 | | | | | | | | 0.46 | | | | | | |
| α-pinene | 0.1 | 2.63 | 1.14 | 5.9 | | | 0.35 | 1.4 | 1.65 | 2.48 | | 1.49 | 0.6 | 1.67 | | |
| α-sinensal | | | | | | | | | 0.35 | 0.49 | | | 0.93 | 5.0 | | |
| α-terpinene | trace | 0.4 | trace | 1.05 | | | | | | | | | | | | |
| α-terpineneol | | | 0.93 | 1.7 | | | | | | | | | | 1.51 | | |
| α-terpineol | trace | 0.4 | | | | | | 0.07 | 2.34 | 3.1 | | 0.14 | 0.2 | 1.1 | | |
| α-terpinolene | | 0.25 | | | | | 1.56 | 2.06 | | | | 0.38 | | 0.1 | | |
| α-thujene | trace | 1.7 | trace | 0.38 | | | 0.04 | 0.11 | | | | 0.11 | | | | |
| α-thujone | | | trace | 0.35 | | | | | | | | | | | | |
| β-bisabolene | 0.2 | 2.0 | | | | | | | | | | | | | | |
| β-caryophyllene | | | | | | | | | | | | 0.21 | | 1.39 | | |
| β-cubebene | | | | | | | | 0.03 | | | | | | 0.37 | | |
| β-eleme | trace | 0.5 | | | | | | 0.1 | | | | | | | | |
| β-elemene | | | | | | | | | | | | 0.17 | | | | |
| β-farnesene | | | | | | | | | | | | 3.64 | | | | |
| β-gurjurene | | | | | | | | 0.0 | | | | | | | | |
| β-myrcene | | | | | | | | 1.88 | 3.76 | 4.41 | | | 3.27 | 4.05 | | |
| β-ocimene | | | | | | | | | | | | 0.27 | | | | |
| β-phellandrene | 0.1 | 4.2 | | | | | | | | | | | | 1.8 | | |
| β-pinene | 0.1 | 15.8 | 0.63 | 31.49 | | | trace | 5.45 | | | | 0.41 | 0.2 | 0.4 | | |
| β-sinensal | | | | | | | | | 0.33 | 0.83 | | | | 0.67 | | |

**Table 4. Chemical composition (%) of citrus peel oils***

| | ***C. limon* (lemon) peel essential oils** | | | | ***C. nobilis* (mandarin orange) essential oil** | | ***C. sinensis* (orange) peel oil** | | | | | | ***C. reticulata* (tangerine) peel oil** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **cold-pressed**[12,13] | | **hydrodistilled**[18] | | **cold-pressed**[14] | | **cold-pressed**[11,12,15,16] | | **hydrodistilled**[44] | | **steam-distilled**[22] | | **hydrodistilled**[21,44] | | | |
| | **Min** | **Max** | **Min** | **Max** | **Min** | **Max** | **Min** | **Max** | **Min** | **Max** | **Min** | **Max** | **Min** | **Max** | | |
| γ-eudesmol | | | | | | | | | | | | | | 1.08 | | |
| γ-munrolene | | | | | | | | | | | | | | 1.16 | | |
| γ-terpinene | trace | 18.57 | 0.04 | 9.96 | | | 0.2 | 1.21 | | | | 3.34 | 0.23 | 2.6 | | |
| δ-amorphene | | | | | | | | 0.05 | | | | | | | | |
| δ-cadinene | | | | | | | | | | 0.32 | | | | 0.88 | | |
| δ-3-carene | | | | | | | | 0.14 | | | | 0.69 | | | | |
| $\Delta^3$-carene | 0.1 | 4.2 | trace | | | | | | 0.78 | 1.03 | | | | | | |

*includes information on various cultivars and/or harvest times
ND – none detected
X – denotes these compounds were present; however, the amount present was not specified

**Table 5. Typical levels of 5-methoxypsoralen (5-MOP)**

| Ingredient | 5-MOP level[26] |
|---|---|
| Tangerine oil cold pressed | 50 ppm |
| Mandarin oil cold pressed | 250 ppm |

**Table 6. Levels of major coumarins and furocoumarins in lemon oil and lime oil**

| Compound | % in Lemon Oil[8] | % in Lime Oil[8] | Photosensitizing Activity[8] |
|---|---|---|---|
| 5-geranoxypsoralen | 0.0387 | 2.2-2.5 | 0 |
| 5-geranoxy-7-methoxycoumrin | 0.0603 | 2.2-5.2 | 0 |
| 5-geranoxy-8-methoxypsoralen | not analyzed | 0.945 | 0 |
| 5,7-dimethoxycoumarin | 0.0295 | 0.464 | 0 |
| 5,8-dimethoxypsoralen | not analyzed | 0.508 | 0 |
| Oxypeucedanin | 0.005-0.073 | 0.0025 | + |
| 5-methoxypsoralen | 0.0001-0.0087 | 0.17-0.33 | ++++ |

**Table 7. Constituents that are established contact allergens in humans, according to the SCCS**

| Constituent | categorized according to number of patients reacting positively and to the number of patients tested (>1000 patients tested, unless indicated as r.t., i.e., rarely tested) [45] |
|---|---|
| β-caryophyllene | ≤10 (oxidized and non-oxidized) |
| carvone | ≤10 (r.t.) |
| citral | 101 to 1000 |
| citronellol | 11-100 |
| coumarin | 101 to 1000 |
| farnesol | 101 to 1000 |
| geraniol | 101 to 1000 |
| linalyl acetate | ≤10 |
| α- and β-pinene | 11-100 |
| (DL)-limonene | 11-100 (non-oxidized); 101 to 1000 (oxidized) |
| terpineol (mixture of isomers)/α-terpineol | ≤10 |
| terpinolene | 11-100 |

**Table 8. Frequency (2014) and concentration of use (2013) according to duration and type of exposure for Citrus-derived ingredients.** [23,24]

| | *# of Uses*[46] | *Max Conc of Use (%)* | *# of Uses* | *Max Conc of Use (%)* | *# of Uses* | *Max Conc of Use (%)* | *# of Uses* | *Max Conc of Use (%)* |
|---|---|---|---|---|---|---|---|---|
| | **Citrus Aurantium Amara (Bitter Orange) Peel Oil** | | **Citrus Aurantium Dulcis (Orange) Peel Oil (listed as Citrus Sinensis (Sweet Orange) Peel Oil in VCRP)** | | **Citrus Grandis (Grapefruit) Peel Oil** | | **Citrus Junos Peel Oil** | |
| **Totals**[1] | **127** | **0.05-2** | **289** | **0.00002-29** | **NR** | **0.00004-0.05** | **6** | **NR** |
| ***Duration of Use*** | | | | | | | | |
| Leave-On | 74 | 0.2-2 | 156 | 0.00038-0.54 | NR | 0.0004-0.0008 | 4 | NR |
| Rinse-Off | 45 | 0.05-0.25 | 110 | 0.00002-29 | NR | 0.00004-0.05 | 2 | NR |
| Diluted for (Bath) Use | 8 | NR | 23 | 0.33 | NR | 0.0014 | NR | NR |
| ***Exposure Type*** | | | | | | | | |
| Eye Area | 2 | NR | 3 | 0.1 | NR | NR | NR | NR |
| Incidental Ingestion | 1 | 0.75 | 4 | NR | NR | NR | NR | NR |
| Incidental Inhalation-Spray | 27[2]; 28[6] | NR | 56[2]; 55[6] | 0.00038[2] | NR | NR | 2 | NR |
| Reported Spray | NR | NR | NR | NR | NR | NR | NR | NR |
| Incidental Inhalation-Powder | 28[6] | 0.9-2[4] | 3[4]; 55[6] | 0.03-0.4[4] | NR | 0.0004[4] | 1 | NR |
| Reported Powder | NR | NR | NR | NR | NR | NR | NR | NR |
| Dermal Contact | 115 | 0.05-2 | 218 | 0.001-0.4 | NR | 0.00004-0.05 | 6 | NR |
| Deodorant (underarm) | NR | NR | 1[2] | NR | NR | NR | NR | NR |
| Reported Spray | NR | NR | NR | NR | NR | NR | NR | NR |
| Reported as Not Spray | NR | NR | NR | NR | NR | NR | NR | NR |
| Hair - Non-Coloring | 11 | NR | 65 | 0.00002-29 | NR | 0.005 | NR | NR |
| Hair-Coloring | NR | NR | NR | NR | NR | NR | NR | NR |
| Nail | NR | NR | 2 | 0.5-0.54 | NR | NR | NR | NR |
| Mucous Membrane | 21 | 0.25-0.75 | 56 | 0.1-0.33 | NR | 0.0014 | 1 | NR |
| Baby Products | 6 | NR | 8 | NR | NR | NR | NR | NR |
| | **Citrus Limon (Lemon) Peel Oil** | | **Citrus Nobilis (Mandarin Orange) Peel Oil** | | **Citrus Reticulata (Mandarin Orange) Peel Oil**[7] | | **Citrus Sinensis Sanguinello (Blood Orange) Peel Oil**[7] | |
| **Totals**[1] | **490** | **0.0001-0.5** | **152** | **0.00005-0.1** | **2** | **NR** | **8** | **NR** |
| ***Duration of Use*** | | | | | | | | |
| Leave-On | 297 | 0.0001-0.5 | 86 | 0.00005-0.1 | 1 | NR | 4 | NR |
| Rinse-Off | 165 | 0.0006-0.001 | 58 | 0.00005-0.03 | 1 | NR | 4 | NR |
| Diluted for (Bath) Use | 28 | 0.012 | 8 | NR | NR | NR | NR | NR |
| ***Exposure Type*** | | | | | | | | |
| Eye Area | 9 | NR | NR | NR | NR | NR | NR | NR |
| Incidental Ingestion | 8 | NR | 1 | 0.0099 | NR | NR | NR | NR |
| Incidental Inhalation-Spray | 84[2]; 96[6] | 0.06[6] | 45[2]; 26[6] | NR | 1[6] | NR | 1[2]; 2 | NR |
| Reported Spray | NR | NR | NR | NR | NR | NR | 1 | NR |
| Incidental Inhalation-Powder | 1[4]; 96[6] | 0.001-0.14[4]; 0.06[6] | 26[6] | 0.00005-0.14 | 1[6] | NR | 2[6] | NR |
| Reported Powder | NR | NR | NR | NR | NR | NR | NR | NR |
| Dermal Contact | 411 | 0.0001-0.5 | 136 | 0.00005-0.1 | 2 | NR | 8 | NR |
| Deodorant (underarm) | NR | NR | NR | NR | NR | NR | NR | NR |
| Reported Spray | NR | NR | NR | NR | NR | NR | NR | NR |
| Reported as Not Spray | NR | 0.002 | NR | NR | NR | NR | NR | NR |
| Hair - Non-Coloring | 67 | NR | 15 | 0.00005-0.03 | NR | NR | NR | NR |
| Hair-Coloring | NR | NR | NR | NR | NR | NR | NR | NR |
| Nail | 3 | 0.0001-0.14 | NR | 0.012 | NR | NR | NR | NR |
| Mucous Membrane | 96 | 0.001-0.012 | 40 | 0.0099 | NR | NR | 4 | NR |
| Baby Products | 8 | NR | NR | NR | NR | NR | NR | NR |

**Table 8. Frequency (2014) and concentration of use (2013) according to duration and type of exposure for Citrus-derived ingredients.** [23,24]

| | # of Uses[46] | Max Conc of Use (%) | # of Uses | Max Conc of Use (%) | # of Uses | Max Conc of Use (%) | # of Uses | Max Conc of Use (%) |
|---|---|---|---|---|---|---|---|---|
| | **Citrus Tangerina (Tangerine) Peel Oil** | | | | | | | |
| **Totals[1]** | **34** | **0.0000013** | | | | | | |
| ***Duration of Use*** | | | | | | | | |
| Leave-On | 18 | 0.0000013 | | | | | | |
| Rinse-Off | 15 | 0.0000013 | | | | | | |
| Diluted for (Bath) Use | 1 | NR | | | | | | |
| ***Exposure Type*** | | | | | | | | |
| Eye Area | NR | NR | | | | | | |
| Incidental Ingestion | 1 | NR | | | | | | |
| Incidental Inhalation-Spray | 5[2]; 8[6] | 0.0000013[2] | | | | | | |
| Reported Spray | 1 | NR | | | | | | |
| Incidental Inhalation-Powder | 8[6] | NR | | | | | | |
| Reported Powder | NR | NR | | | | | | |
| Dermal Contact | 26 | NR | | | | | | |
| Deodorant (underarm) | NR | NR | | | | | | |
| Reported Spray | NR | NR | | | | | | |
| Reported as Not Spray | NR | NR | | | | | | |
| Hair - Non-Coloring | 7 | 0.0000013 | | | | | | |
| Hair-Coloring | NR | NR | | | | | | |
| Nail | NR | NR | | | | | | |
| Mucous Membrane | 7 | NR | | | | | | |
| Baby Products | NR | NR | | | | | | |

NR = Not reported.

1. Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.
2. It is possible these products may be sprays, but it is not specified whether the reported uses are sprays.
3. Use in a spray product has been reported in response to a survey conducted by the Council.
4. It is possible these products may be powders, but it is not specified whether the reported uses are powders.
5. Use in a powder product has been reported in response to a survey conducted by the Council.
6. Not specified whether a powder or a spray, so this information is captured for both categories of incidental inhalation.
7. Not listed as an INCI name; included because of similarity

**Table 9. Ingredients that are not reported to be in use**

Citrus Aurantifolia (Lime) Peel Oil
Citrus Aurantium Currassuviensis Peel Oil
Citrus Clementina Peel Oil
Citrus Iyo Peel Oil
Citrus Medica Vulgaris Peel Oil
Citrus Reticulata (Tangerine) Peel Oil
Citrus Tachibana/Reticulata Peel Oil

**Table 10 . Genotoxicity**

| Test Article | Concentration/Dose | Procedure | Results | Reference |
|---|---|---|---|---|
| | | **IN VITRO** | | |
| lemon oil in ethanol | 10-5000 µg/plate | Reverse mutation assay using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2uvrA, with and without S9 metabolic activation. | Toxicity was observed in all test strains except WP2uvrA with and without S9; no significant dose-related increases in the number of revertant colonies in any test strain at any dose level, with or without metabolic activation; controls yielded expected results; lemon oil was not mutagenic in this assay. | [3] |
| lemon oil in ethanol | 40-100 µg/ml | Cell mutation assay in mouse lymphoma L5178Y TK+/-cells in accordance with OECD guideline 476 in 2 independent experiments; with and without S9 metabolic activation | No significant increases in the mutation frequency were observed in either experiment, with or without S9 activation; controls yielded the expected results; lemon oil did not induce gene mutations in mouse lymphoma cells | [3] |
| lemon oil in ethanol | up to 0.125 mg/ml | Chromosome aberration study using Chinese hamster lung fibroblasts (CHL) in accordance with OECD guideline 473, without metabolic activation, 100 metaphases examined | The incidence of polyploidy cells at 24 h post-treatment was 1.0%, and the incidence of cells with structural chromosome aberrations at 24 h after treatment was 2.0%; the test material did not significantly induce chromosomal aberrations in CHL cells; lemon oil was not considered clastogenic. | [3] |
| sweet orange oil in ethanol | 1-5000 µg/plate | Reverse mutation assay using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2uvrA, with and without S9 metabolic activation. | Cytotoxicity was observed in all test strains except WP2uvrA with and without S9; no significant dose-related increases in the number of revertant colonies in any test strain at any dose level, with or without metabolic activation; controls yielded expected results; sweet orange oil was not mutagenic in this assay. | [4] |
| sweet orange oil in ethanol | 40-100 µg/ml | Cell mutation assay in mouse lymphoma L5178Y TK+/-cells in accordance with OECD guideline 476 in 2 independent experiments; with and without S9 metabolic activation | The test material did not induce a significant increase in the mutation frequency in both experiments, with or without metabolic activation; sweet orange oil did not induce gene mutations in mouse lymphoma cells | [4] |
| sweet orange oil in ethanol | up to 0.125 mg/ml | Chromosome aberration study using Chinese hamster lung fibroblasts (CHL) in accordance with OECD guideline 473, without metabolic activation, 100 metaphases examined | The incidence of polyploidy cells at 48 h post-treatment was 1.0%, and the incidence of cells with structural chromosome aberrations at 48 h after treatment was 1.0%; the test material did not significantly induce chromosomal aberrations in CHL cells; sweet orange oil was not considered clastogenic. | [4] |

**Table 11. Dermal irritation studies for Citrus-derived ingredients**

| Test Article | Concentration/Dose | Test Population | Procedure | Results | Reference |
|---|---|---|---|---|---|
| **NON-HUMAN** | | | | | |
| lemon oil | 5 ml/kg1 | 6 New Zealand White rabbits | acute dermal toxicity limit test scored under the Draize method; 24-h occlusive patches on intact and abraded skin | irritating | [3] |
| orange oil, cold pressed | 0.5 ml of undiluted material | 3 male albino rabbits | skin irritation study conducted according to OECD guideline 404; semi-occluded patch for 4 h | mean erythema/eschar scores were 2.0, 1.7, and 2.0; mean edema scores were 2.0, 1.3, and 1.3; irritating to skin | [4] |
| mandarin peel oil, expressed (described as *Citrus reticulata*) | 5 mg/kg | 7 rabbits | 24-h occlusive, single dose study | slight erythema and edema | [10] |
| mandarin peel oil, expressed (described as *Citrus reticulata*) | not reported | hairless mice and miniature swine; details not provided | open patch tests; details not provided | 2 of 3 samples were irritating | [10] |
| **HUMAN** | | | | | |
| lemon oil | 0.3%, 2% or 20%; multiple vehicles | 34 subjects at 0.3%, 30 subjects at 2%, and 35 subjects at 20% | 24-72 h occlusive patch tests | no irritation at 0.3% and 20%, 1 + reaction at 2% | [36] |
| mandarin peel oil, expressed (described as *Citrus reticulata*) | 8% in petrolatum | 5 subjects | 48 h closed patch test; details not provided | no irritation | [10] |

**Table 12. Sensitization studies for Citrus-derived ingredients**

| Test Article | Concentration/Dose | Test Population | Procedure | Results | Reference |
|---|---|---|---|---|---|
| **HUMAN - Patch** | | | | | |
| bitter orange oil | 2% in paraffin | 200 patients with dermatitis tested with 35 essential oils plus an additional 50 patients with balsam sensitivity | sensitization patch study, details not provided | 6 positive reactions | [37] |
| lemon oil | 2% in paraffin | 200 patients with dermatitis tested with 35 essential oils plus an additional 50 patients with balsam sensitivity | sensitization patch study, details not provided | 4 positive reactions, details not provided | [37] |
| lemon oil | not reported | 100 subjects | Marzulli-Maibach sensitization technique; open patches | 2% of the subjects had a positive skin reaction at the first reading after challenge, no reactions were noted at the 48 and 72 h readings, study concluded the test material was not sensitizing | [3] |
| mandarin oil, expressed (described as *Citrus reticulate*) | 8% in petrolatum | 25 subjects | maximization study, details not provided | not sensitizing | [10] |
| sweet orange oil | 2% in paraffin | 200 patients with dermatitis tested with 35 essential oils plus an additional 50 patients with balsam sensitivity | sensitization patch study, details not provided | 3 positive reactions, details not provided | [37] |
| sweet orange oil | not reported | 100 subjects | Marzulli-Maibach sensitization technique; open patches | 4% of the subjects had positive skin reaction at the first reading after challenge, no reactions were noted at the 48 and 72 h readings, study concluded the test material was not sensitizing | [4] |

**Table 13. Photosensitization and phototoxicity studies**

| Test Article | Concentration/Dose* | Test Population | Procedure | Results | Reference |
|---|---|---|---|---|---|
| **ALTERNATIVE STUDIES** | | | | | |
| sweet orange oil, including deterpenated kind | concentrations not reported; tested in PBS, ethanol, or DMSO with samples from 3 suppliers | 3T3 Balb/c fibroblasts | -3T3 neutral red uptake phototoxicity test<br>-light source was a doped mercury-metal halide lamp, filtered with 50% transmission at 335 nm to diminish UVB | borderline phototoxic, positive phototoxic results observed more frequently with the vehicles PBS and ethanol with certain supplied samples | 38 |
| lemon oil, including deterpenated kind | same as above | same as above | same as above | borderline phototoxic, positive phototoxic results observed in all 3 vehicles, but were more prominent in the deterpenated sample | 38 |
| sweet orange oil, including deterpenated kind | up to 3.16% in water with samples from 3 suppliers | same as above | same as above | potential for phototoxicity observed | 38 |
| lemon oil, including deterpenated kind | same as above | same as above | same as above | cytotoxicity observed with deterpenated lemon oil; potential for phototoxicity observed | 38 |
| **NON-HUMAN** | | | | | |
| lime oil, distilled (psoralen-free) | undiluted; 20 µl | hairless mice, 6/grp | - a single dose was applied to a 2 $cm^2$ area on the back; animals were exposed to irradiation 30 min after dosing<br>- one group was exposed to a compact-arc xenon lamp for 2 min (wavelengths <295 nm or 320-280 nm excluded)<br>- one group was exposed to a long-arc xenon lamp for 40 min at a distance of 1 m; the weighted energy was 0.1667 $W/m^2$<br>- one group was exposed to 4 fluorescent black light lamps (UVB eliminated) for 1 h at an integrated UVA intensity of 3 $W/m^2$<br>-positive controls were treated with 0.01% 8-methoxypsoralen in methanol; negative controls with an appropriate vehicle<br>- test sites were examined 4, 24, 48, 72, and 96 h after exposure | - a phototoxic response was not observed with any of the light sources | 40 |
| lime oil, distilled (psoralen-free) | undiluted; 20 µl | miniature swine, 2/grp | as above | - a phototoxic response was not observed with any of the light sources | 40 |
| lime oil, expressed | undiluted and diluted; 20 µl | hairless mice, 6/grp | as above | - a phototoxic response was observed with all three light sources<br>- the lowest phototoxic concentration was 15% | 40 |
| lime oil, expressed | undiluted and diluted; 20 µl | miniature swine, 2/grp | as above | - a phototoxic response was observed with all three light sources<br>- the lowest phototoxic concentration was 30% | 40 |
| grapefruit oil | undiluted; 20 µl | 6 hairless mice and 2 miniature swine | - as above | not photosensitizing | 40 |
| lime oil distilled (psoralen-free) | undiluted; 20 µl | 6 hairless mice and 2 miniature swine | - as above | not photosensitizing | 40 |
| lime oil; expressed and rectified | undiluted; 20 µl | 6 hairless mice and 2 miniature swine | - as above | not photosensitizing | 40 |
| lime oil Persian Florida; expressed and rectified | undiluted; 20 µl | 6 hairless mice and 2 miniature swine | - as above | not photosensitizing | 40 |

**Table 13. Photosensitization and phototoxicity studies**

| Test Article | Concentration/Dose* | Test Population | Procedure | Results | Reference |
|---|---|---|---|---|---|
| mandarin oil | undiluted; 20 µl | 6 hairless mice and 2 miniature swine | - as above | not photosensitizing | [40] |
| mandarin oil, Italian | undiluted; 20 µl | 6 hairless mice and 2 miniature swine | - as above | not photosensitizing | [40] |
| oil of lemon, California | undiluted; 20 µl | 6 hairless mice and 2 miniature swine | - as above | not photosensitizing | [40] |
| oil of lemon, distilled | undiluted; 20 µl | 6 hairless mice and 2 miniature swine | - as above | not photosensitizing | [40] |
| oil mandarin, Italian | undiluted; 20 µl | 6 hairless mice and 2 miniature swine | - as above | not photosensitizing | [40] |
| oil of tangerine | undiluted; 20 µl | 6 hairless mice and 2 miniature swine | - as above | not photosensitizing | [40] |
| orange oil; cold pressed | undiluted; 20 µl | 6 hairless mice and 2 miniature swine | - as above | not photosensitizing | [40] |
| California lemon oil | undiluted; 20 µl | 6 hairless mice and 2 miniature swine | - as above | - a phototoxic response was observed | [40] |
| Italian lemon oil | undiluted; 20 µl | 6 hairless mice and 2 miniature swine | - as above | - a phototoxic response was observed | [40] |
| oil lemon, Greek; cold pressed | undiluted; 20 µl | 6 hairless mice and 2 miniature swine | - as above | - a phototoxic response was observed | [40] |
| oil lemon, Italian | undiluted; 20 µl | 6 hairless mice and 2 miniature swine | - as above | - a phototoxic response was observed | [40] |
| oil lemon, IC (not defined) | undiluted; 20 µl | 6 hairless mice and 2 miniature swine | - as above | - a phototoxic response was observed | [40] |
| lime oil expressed | undiluted; 20 µl | 6 hairless mice and 2 miniature swine | - as above | - a phototoxic response was observed | [40] |
| oil limes Persian | undiluted; 20 µl | 6 hairless mice and 2 miniature swine | - as above | - a phototoxic response was observed | [40] |
| oil limes, expressed and rectified | undiluted; 20 µl | 6 hairless mice and 2 miniature swine | - as above | - a phototoxic response was observed | [40] |
| lime oil, expressed and rectified | undiluted; 20 µl | 6 hairless mice and 2 miniature swine | - as above | - a phototoxic response was observed | [40] |
| bitter orange oil | undiluted; 20 µl | 6 hairless mice and 2 miniature swine | - as above | - a phototoxic response was observed | [40] |
| lemon oil from multiple regional sources | 20, 50, or 100% in ethanol; 0.02 ml | albino guinea pigs | - the oil was applied to the shaved back of the animals<br>- the animals were then exposure to UVA radiation (320-400 nm, 13 J/cm$^2$)<br>- erythema was evaluated 24, 48, and 72 h after irradiation<br>- the samples were then fractionated subsequent phototoxicity testing of the isolated components was performed | -concentrations of 50% and 100% elicited phototoxicity in most of the samples tested<br>- lemon oils from different regions had different phototoxicity potencies<br>-oxypeucedanin and 5-methoxypsoralen (furocoumarins) were identified as phototoxic | [41] |

**Table 13. Photosensitization and phototoxicity studies**

| Test Article | Concentration/Dose* | Test Population | Procedure | Results | Reference |
|---|---|---|---|---|---|
| | | | **HUMAN** | | |
| bitter orange peel oil | undiluted; 5 µl/cm$^2$ | 8 subjects | - an occlusive patch was applied to a 2 cm x 2 cm area<br>- 1 cm site on each subject was exposed to visible light t of 20 J/cm$^2$ UVA<br>- the test sites were scored after 24 and 48 h | all subjects reacted (details not provided) | 30 |
| lemon oil, including deterpenated kind | up to 1% in water from samples from 3 supplier; dose not provided | 5 female subjects | -2 occlusive 10 mm diameter Finn Chambers on both sides of the lower back<br>-exposure time to test material was 1 h<br>-irradiation immediately to 1 site after patch removal at a dose of 5 J/cm$^2$as measured in the UVA range<br>-test sites scored after 24, 48, and 72 h<br>-light source was a doped mercury-metal halide lamp, filtered with 50% transmission at 335 nm to diminish UVB | -phototoxic reactions concurrent with an irritation reactions were observed in lemon oil at 1% in 4/5 subjects up to 72 h after irradiation<br>-phototoxic reactions were observed in deterpenated lemon oil at 0.1% in 2/5 subjects at 48 and 72 h after irradiation<br>-no reactions were observed at concentrations of 0.1% or lower in lemon oil and 0.01% in deterpenated lemon oil | 38 |
| sweet orange oil, including deterpenated kind | same as above | same as above | -same as above | -phototoxic reaction were observed in orange oil at 1% in 3/5 subjects at 24 h and 2/5 subjects at 48 and 72 h after irradiation<br>-no reactions were observed at concentrations of 0.1% or lower in orange oil or 0.1% and 0.01% in deterpenated orange oil | 38 |
| lime oil, distilled | undiluted | 10 Caucasian subjects | - a single dose was applied to a 2 cm$^2$ area on the back<br>- 30 min after dosing, subjects were exposed to sunlight for 30 min or a compact-arc xenon lamp for 2 min (wavelengths <295 nm or 320-280 nm excluded)<br>-positive controls were treated with 0.01% 8-methoxypsoralen in methanol; negative controls with an appropriate vehicle<br>- test sites were examined 4, 24, 48, 72, and 96 h after exposure | no phototoxic response was observed | 40 |
| lime oil, expressed | undiluted and diluted; 20 µl | 10 Caucasian subjects | - a single dose was applied to a 2 cm$^2$ area on the back<br>- 30 min after dosing, 1 treated site and the control untreated site were exposed to sunlight for 30 min, a compact-arc xenon lamp for 2 min (wavelengths <295 nm or 320-280 nm excluded), or 4 fluorescent black light lamps (UVB eliminated) for 1 h at an integrated UVA intensity of 3 W/m$^2$<br>-positive controls were treated with 0.01% 8-methoxypsoralen in methanol; negative controls with an appropriate vehicle<br>- test sites were examined 4, 24, 48, 72, and 96 h after exposure | - a phototoxic response was observed with all three light sources<br>- the lowest phototoxic concentration with the simulated light sources was 30% | 40 |

* the solvent is specified when known

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# OPINION OF THE SCIENTIFIC COMMITTEE ON COSMETIC PRODUCTS AND NON-FOOD PRODUCTS INTENDED FOR CONSUMERS

CONCERNING

# AN INITIAL LIST OF PERFUMERY MATERIALS WHICH MUST NOT FORM PART OF COSMETIC PRODUCTS EXCEPT SUBJECT TO THE RESTRICTIONS AND CONDITIONS LAID DOWN

adopted by the SCCNFP during the 18th Plenary meeting
of 25 September 2001

## 1. Terms of Reference

In recent years there has been concern on the safety of fragrance (perfumery) materials. Dermatologists have highlighted the frequency of allergic contact dermatitis from perfumes.

Under current legislation, fragrance materials do not fall under all the requirements of Directive 76/768/EEC on cosmetic products. The 6th Amendment (93/35/EEC) provides for the labelling of ingredients on cosmetic products. However, it is not a requirement to label fragrance constituents on the packaging of cosmetic products, current legislation requires only the word *parfum*.

In response to growing concern over this issue, the Commission was asked for positive actions with respect to legislative measures on fragrance materials.

## 2. Mandate

The SCCNFP has been asked to respond to the following questions :

1. Does the SCCNFP agree to the inclusion of all IFRA restricted materials in the Annex III (List of substances which cosmetic products must not contain except subject to restrictions and conditions laid down)? Are the permitted levels recommended by IFRA suitable for use in the Cosmetics Directive 76/768/EEC ?

2. Does the SCCNFP agree that all materials that IFRA recommend should not be used as fragrance compounds are included in Annex II (List of substances which must not form part of the composition of cosmetic products)?

3. It is proposed that all known fragrance allergens are labelled on cosmetics if used in the products. Does the SCCNFP agree to this proposal? If so :

   - Which chemicals fall under this classification ?
   - Is there a maximum concentration of each chemical permissible without the requirement for labelling ?

4. Restrictions are proposed for the 3 most common fragrance allergens (cinnamic aldehyde, isoeugenol, hydroxycitronellal). Does the SCCNFP agree to restriction on the use of common fragrance allergens (Annex III listing)? If so :

   - Which fragrance materials should be subject to restrictions?
   - What are the conditions for restrictions (maximum concentration, fields of applications, etc) ?

Obviously, in response to each of the questions listed above, a scientific justification will be necessary.

## 3. Strategy of the SCCNFP

The SCCNFP has considered that this mandate can be usefully divided into two sections (Interim position on Fragrance allergy, document n° SCCNF/0202/99 adopted by the SCCNFP during the 8th Plenary meeting of 23 June 99) :

1. Identification of those fragrance ingredients, which are of concern as allergens for the consumer. Recommendations on informing the consumer of the presence of important allergens to permit the consumer with a known fragrance allergy a means to avoid contact with an allergen. An opinion as to whether such identification can be related to concentrations present in a product when elicitation levels are known.

2. An opinion on the adoption of industry prohibited substances into Annex 2 and adoption of industry restricted substances into Annex 3. Considerations as to whether the concentration limits or other restrictions suggested by industry can be supported or need to be changed if there is such inclusion in Annex 3. Whether there are additional substances which should be subject to inclusion in an Annex.

An opinion concerning fragrance allergy was adopted by the SCCNFP during the 10th Plenary meeting of 8 December 1999 (doc. n° SCCNFP/0017/98 final). During its 12th plenary meeting of 3 May 2000, the SCCNFP adopted an opinion on an initial list of perfumery materials which must not form part of fragrance compounds used in cosmetic products (doc. n° SCCNFP/0320/00 final).

The current opinion consists of the adoption of an initial list of perfumery materials to be included in Annex III - List of substances which cosmetic products must not contain except subject to restrictions and conditions laid down - to Directive 76/768/EEC.

The opinion is based on information submitted as ‘monographs’ (synopses) on behalf of industry.

## 4. Opinion

On the basis of the available information and assessment of the cutaneous toxicity of the substances tabulated, it is the recommendation of the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) that these substances may be used as ingredients in cosmetic products only under the conditions and restrictions specified in the attached table.

Additional substances will be discussed for possible inclusion at a later date.

Table 1 : List of perfumery materials which must not form part of cosmetic products except subject to the restrictions and conditions laid down

| N° | Substance name | Restriction and condition |
|---|---|---|
| 1 | *Abies alba oil from cones (Abies Alba Mill.)*<br>CAS n° : 8021-27-0<br><br>*Abies alba oil from needles (Abies Alba Mill.)*<br>CAS n° : 8021-27-0<br><br>*Abies sachalinensis oil*<br>CAS n° : Unkown<br><br>*Fir balsam (Abies balsamea (L.) Mill)*<br>CAS n° : 8021-28-1<br><br>*Fir needle oil (Abies sibirica)*<br>CAS n° : 8021-29-2<br><br>*Fir needle oil, Canadian (Abies balsamea)*<br>CAS n° : 8021-28-1<br><br>*Pine needle, dwarf, oil (Pinus mugo turra var. pumilio (Haenke) Zenari)*<br>CAS n° : 8000-26-8<br><br>*Pine needle oil (Abies spp.)*<br>CAS n° : 8021-29-2<br><br>*Pine scotch oil (Pinus sylvestris L.)*<br>CAS n° : 8023-99-2<br><br>*Pinus nigra oil*<br>CAS n° : 90082-74-9<br><br>*Turpentine gum (Pinus spp.)*<br>CAS n° : 9005-90-7<br><br>*Turpentine oil*<br>CAS n° : 8006-64-2<br><br>*Turpentine oil rectified*<br>CAS n° : 8006-64-2<br><br>*Turpentine, steam distilled (Pinus spp.)*<br>CAS n° : 8006-64-2 | Essential oils and isolates derived from the Pinacea family, including Pinus and Abies genera, should only be used when the level of peroxides is kept to the lowest practicable level, for instance by adding antioxidants at the time of production. Such products should have a peroxide value of less than 10 millimoles peroxide per liter. Based on the published literature mentioning sensitising properties when containing peroxides (Food and Chemical Toxicology 11,1053(1973); 16,843(1978); 16,853(1978). |
| 2 | Acetyl hexamethyl indan<br><br>CAS n° : 15323-35-0 | For applications on areas of skin exposed to sun, excluding bath preparations, soaps and other rinse-off products, limit use to 2%in the finished cosmetic product. Based on the phototoxic potential showing no effects at 10% in human photo-toxicity tests (IFRA guidelines). |

| N° | Substance name | Restriction and condition |
|---|---|---|
| 3 | *Allyl butyrate*<br>CAS n° : 2051-78-7<br><br>*Allyl cinnamate*<br>CAS n° : 1866-31-5<br><br>*Allyl cyclohexaneacetate*<br>CAS n° : 4728-82-9<br><br>*Allyl cyclohexanepropionate*<br>CAS n° : 2705-87-5<br><br>*Allyl heptanoate*<br>CAS n° : 142-19-8<br><br>*Allyl hexanoate*<br>CAS n° : 123-68-2<br><br>*Allyl isovalerate*<br>CAS n° : 2835-39-4<br><br>*Allyl octanoate*<br>CAS n° : 4230-97-1<br><br>*Allyl phenoxyacetate*<br>CAS n° : 7493-74-5<br><br>*Allyl phenylacetate*<br>CAS n° : 1797-74-6<br><br>*Allyl 3,5,5-trimethylhexanoate*<br>CAS n° : 71500-37-3 | Use only when the level of free allyl alcohol in the ester is less than 0.1%. Based on the delayed irritant potential of allyl alcohol (Food and Chemical Toxicology 15,611(1977)). |
| 4 | *Allyl heptine carbonate*<br><br>CAS n° : 73157-43-4 | Should not be used such that the level in the finished cosmetic products exceeds 0.002 %. Based on test results showing the absence of sensitising reactions for allyl heptine carbonate at low concentrations in a test on humans. This material should not be used in combination with any other 2-alkynoic acid ester (e.g. methyl heptine carbonate). (IFRA guidelines). |
| 5 | *Amylcyclopentenone*<br><br>CAS n° : 25564-22-1 | Should not be used such that the level in the finished cosmetic product exceeds 0.1 %. Based on test results showing sensitisation at 10% and no sensitisation at 1 %. (IFRA guidelines). |

| N° | Substance name | Restriction and condition |
|---|---|---|
| 6 | *Angelica root oil (Angelica archangelica L.)*<br>CAS n° : 8015-64-3<br><br>*Bergamot oil*<br>CAS n° : 8007-75-8<br><br>*Grapefruit oil, expressed (Citrus paradisi Macf.)*<br>CAS n° : 8016-20-4<br><br>*Lemon oil*<br>CAS n° : 8008-56-8<br><br>*Lemon oil, cold pressed, California type*<br>CAS n° : 8008-56-8<br><br>*Lemon oil, cold pressed, desert type*<br>CAS n° : 8008-56-8<br><br>*Lime oil, cold pressed, Mexican*<br>CAS n° : 8008-26-2<br><br>*Lime oil, expressed*<br>CAS n° : 8008-26-2<br><br>*Lime oil expressed rectified*<br>CAS n° : 8008-26-2<br><br>*Orange peel oil, bitter (Citrus aurantium L.)*<br>CAS n° : 68916-04-1<br><br>*Rue oil (Ruta graveolens L.)*<br>CAS n° : 8014-29-7 | May be used in cosmetic products, provided that the total concentration of furocoumarin-like substances in the finished cosmetic product do not exceed 1ppm. |
| 7 | *Balsam oil, Peru (Myroxylon pereirae Klotzsch)*<br>CAS n° : 8007-00-9<br><br>*Balsam absolute, Peru*<br>CAS n° : 8007-00-9<br><br>*Balsam anhydrol, Peru*<br>CAS n° : 8007-00-9 | Extracts and distillates of Peru balsam (the exudation from Myroxylon pereirae (Royle) Klotzsch) should not be used such that the total level exceeds 0.4% in cosmetic products. Based on a wide variety of test results on the sensitising potential of Peru balsam and its derivatives. |
| 8 | *p-tert-Butyldihydro-cinnamaldehyde*<br><br>CAS n° : 18127-01-0 | Should not be used such that the level in the finished cosmetic product exceeds 0.6%. Based on test results showing sensitising potential and no sensitisation reactions when tested at 6%. (IFRA guidelines). |

| N° | Substance name | Restriction and condition |
|---|---|---|
| 9 | Cinnamal<br>CAS n° : 104-55-2<br><br>*Cinnamic aldehyde-methyl anthranilate (Schiff base)*<br>CAS n° : 94386-48-8 | The concentration of Cinnamic aldehyde in the finished cosmetic product should not exceed 0.1%. |
| 10 | *Cassia oil*<br>CAS n° : 8007-80-5<br><br>*Cinnamon bark oil*<br>CAS n° : 8015-91 –6 | The prime allergen is Cinnamic aldehyde. The concentration of Cinnamic aldehyde in the finished cosmetic product should not exceed 0.1%. |
| 11 | Cinnamyl alcohol<br><br>CAS n° : 104-54-1 | Should not be used such that the level in finished cosmetic products exceeds 0.8%. Based on test results showing sensitising potential. (IFRA guidelines). |
| 12 | *Cumin oil*<br><br>CAS n° : 8014-13-9 | For applications on areas of skin exposed to sun, excluding bath preparations, soaps and other wash-off products, limit cumin oil to 0.4% in the finished cosmetic product. Based on the photo-toxicity of cumin oil, the observed no-effect level of 25% on the skin of the hairless mouse and on the no-effect level of 5% in tests with humans. |
| 13 | *cis-.alpha.-Damascone*<br>CAS n° : 23726-94-5<br><br>*trans-. beta.-Damascone*<br>CAS n° : 23726-91-2<br><br>*Isodamascone*<br>CAS n° : 39872-57-6<br><br>*1-(2,6,6-Trimethylcyclohexa-1,3-dienyl)-2-buten-1-one*<br>CAS n° : 23698-85-7<br><br>*1-(2,6,6-Trimethyl-3-cyclohexen-1-yl)-2-buten-l-one*<br>CAS n° : 57378-68-4<br><br>*1-(2,6,6-Trimethyl-l-cyclohexen-1-yl)-2-buten-l-one*<br>CAS n° : 23726-92-3<br><br>*1-(2,6,6-Trimethyl-2-cydohexen-1-yl)-2-buten-l-one*<br>CAS n° : 43052-87-5 | Should not be used as fragrance ingredients such that the total level in finished cosmetic products exceeds 0.02%, individually or in combination. Based on test data showing sensitising potential for these materials and on evidence of cross-reactivity. (IFRA guidelines) |
| 14 | *trans-Hexen-2-al*<br><br>CAS n° : 6728-26-3 | Should not be used such that the level in finished cosmetic products exceeds 0.002%. Based on test results of RIFM showing sensitisation reactions at 0.2% and no sensitisation reactions when tested at 0.02% (IFRA guidelines). |

| N° | Substance name | Restriction and condition |
|---|---|---|
| 15 | Hydroxycitronellal<br><br>CAS n° : 107-75-5 | Should not be used such that the level in finished cosmetic products exceeds 1.0%. Based on a review of a large number of animal and human test data (IFRA guidelines). |
| 16 | Isoeugenol<br><br>CAS n° : 97-54-1 | Should not be used such that the level in finished cosmetic products exceeds 0.02%. Based on test results showing sensitising potential (IFRA guidelines). |
| 17 | *d-Limonene*<br>CAS n° : 5989-27-5<br><br>*l-Limonene*<br>CAS n° : 5989-54-8<br><br>dl-Limonene (racemic)<br>CAS n° : 138-86-3 | Limonene and natural products containing substantial amounts of it, should only be used when the level of peroxides is kept to the lowest practical level, for instance by adding antioxidants at the time of production. Such products should have a peroxide value of less than 20 millimoles peroxide per liter. (IFRA guidelines). |
| 18 | *p-Mentha-1,8-dien-7-al*<br><br>CAS n° : 2111-75-3 | Should not be used such that the level in finished cosmetic products exceeds 0.1%. Based on test results showing sensitisation (IFRA guidelines). |
| 19 | *Menthadiene-7-methyl formate*<br><br>CAS n° : 68683-20-5 | Should not be used such that the level in finished cosmetic products exceeds 0.1 %. Based on tests showing sensitisation (IFRA guidelines). |
| 20 | *Methoxy dicyclopentadiene carboxaldehyde*<br><br>CAS n° : 86803-90-9 | Should not be used such that the level in finished cosmetic products exceeds 0.5%. Based on test results showing sensitisation potential (IFRA guidelines). |
| 21 | *Methyl N-methylanthranilate*<br><br>CAS n° : 85-91-6 | For applications on areas of the skin exposed to sunlight, excluding bath preparations, soaps and other wash-off products, limit to 10**%** in the finished cosmetic product. Based on the phototoxic potential and on the observed no-effect level of approximately 2 mg/cm$^2$ of the hairless mouse (Food and Chemical Toxicology 17, 273 (1979)). |
| 22 | *3-Methyl-2(3)-nonenenitrile*<br><br>CAS n° : 53153-66-5 | Should not be used such that the level in finished cosmetic products exceeds 0.2%. Based on test results showing sensitisation (Food Chemical Toxicology 20, 757 (1982)). |
| 23 | *Methyl octine carbonate*<br>CAS n° : 111-80-8<br><br>*Methyl heptine carbonate*<br>CAS n° : 111-12-6 | Should not be used such that the level in finished cosmetic products exceeds 0.002%.<br><br>When used alone level in the finished cosmetic products should not exceed 0.01%.<br><br>When present in combination, the combined level in the finished product should not exceed 0.01% of which methyl heptine carbonate should not be more than 0.002% |

| N° | Substance name | Restriction and condition |
|---|---|---|
| 24 | *Oakmoss absolute (Evernia spp.)*<br>CAS n° : 9000-50-4<br><br>*Oakmoss resinoid (Evernia spp.)*<br>CAS n° : 9000-50-4<br><br>*Treemoss absolute (Usnea spp.)*<br>CAS n° : 68648-41-9<br><br>*Treemoss concrete (Usnea spp.)*<br>CAS n° : 68648-41-9<br><br>*Treemoss resinold (Usnea spp.)*<br>CAS n° : 868648-41-9 | Should not be used such that the level in finished cosmetic products exceeds 0.1%. The maximum concentration of Oakmoss and Treemoss in combination in the finished cosmetic product should not exceed 0.1%.<br><br>The allergenic fractions in these species have not been fully elucidated nor has the relative contribution of the allergenic fractions between the species. Information needs to be obtained on the nature and the importance of the respective allergenic fractions at which time a review of the acceptance of the restriction in levels and relative proportions of the species will be undertaken. |
| 25 | *1-Octen-3-yl acetate*<br><br>CAS n° : 2442-10-6 | Should not be used such that the level in finished cosmetic products exceeds 0.3%. Based on test results showing sensitising potential (IFRA guidelines). |
| 26 | *3-Propylidenephthalide*<br><br>CAS n° : 17369-59-4 | Should not be used such that the level in finished cosmetic products exceeds 0.01 %. Based on test results showing sensitisation potential (IFRA guidelines). |
| 27 | *2,4,6-Trimethyl-3-cyclohexene-1-methanol*<br><br>CAS n° : 68527-77-5 | Should not be used such that the level in finished cosmetic products exceeds 0.5%. Based on test results showing sensitisation potential (IFRA guidelines). |
| 28 | *Verbena absolute*<br><br>CAS n° : 8024-12-2 | Verbena absolute obtained from Lippia citriodora Kunth should not be used such that the level in finished cosmetic products exceeds 0.2%. Based on test results showing sensitisation (IFRA guidelines). |
| | | |

EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Public Health and Risk Assessment
**C7 - Risk assessment**

**SCIENTIFIC COMMITTEE ON CONSUMER PRODUCTS**

# SCCP

# Opinion on

# Furocoumarins in cosmetic products

Adopted by the SCCP
during the 6th plenary of 13 December 2005

## TABLE OF CONTENTS

1. BACKGROUND …… 3

2. TERMS OF REFERENCE …… 4

3. OPINION …… 4

4. DISCUSSION …… 7

5. CONCLUSION …… 9

6. MINORITY OPINION …… 9

7. REFERENCES …… 9

8. ACKNOWLEDGEMENTS …… 9

## 1. BACKGROUND

Commission Directive 95/34/EC July 10, 1995 adopted an amendment to Annex II, Annex to Cosmetic Directive under reference number 358 as follows: "Furocoumarins (e.g. trioxysalan, 8-methoxypsoralen, 5-methoxypsoralen) except for normal content in natural essences used. In sun protection and in bronzing products, furocoumarins shall be below 1 mg/kg."

The technical adaptation was based on an opinion adopted by the Scientific Committee on Cosmetology (SCC) in 1990. Furocoumarins are recognized to be photomutagenic and photo-carcinogenic. International Agency for Research on cancer (IARC) has classified 5-MOP and 8-MOP plus ultraviolet radiation in group 2A (probably carcinogenic to humans) and in group 1 (carcinogenic to human), respectively.

The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) adopted an "Initial List of Perfumery Materials which must not form part of Cosmetic Products except subject to the restrictions and conditions laid down" (SCCNFP/0392/00, final, adopted by the SCCNFP during the 18th Plenary meeting of 25 September 2001). The opinion is based on information submitted as 'monographs' (synopses) on behalf of industry. On the basis of the available information and assessment of the cutaneous toxicity of the substances tabulated in its opinion, it is the recommendation of the SCCNFP that these substances may be used as ingredients in cosmetic products only under the conditions and restrictions specified in the table attached in its opinion.

This opinion under entry nr 6 mentions that the following 11 essential oils: *Angelica root oil (Angelica archangelica L.)* CAS n° 8015-64-3; *Bergamot oil* CAS n° 8007-75-8; *Grapefruit oil, expressed (Citrus paradisi Macf.)* CAS n° 8016-20-4; *Lemon oil* CAS n° 8008-56-8; *Lemon oil, cold pressed, California type* CAS n° 8008-56-8; *Lemon oil, cold pressed, desert type* CAS n° 8008-56-8; *Lime oil, cold pressed, Mexican* CAS n° 8008-26-2; *Lime oil, expressed* CAS n° 8008-26-2; *Lime oil expressed rectified* CAS n° 8008-26-2; *Orange peel oil, bitter (Citrus aurantium L.)* CAS n° 68916-04-1; *Rue oil (Ruta graveolens L.)* CAS n° 8014-29-7 "may be used in cosmetic products, provided that the total concentration of furocoumarin-like substances in the finished cosmetic product does not exceed 1 ppm."
These plant extracts are often used as ingredients in fragrances and cosmetic products.

Submission I from European Flavour & Fragrance Association (EFFA) was submitted to the SCCNFP in November 2002.

Submission II was received in August 2003

The SCCNFP adopted during the 26th plenary meeting December 2003 an opinion concerning Furocoumarins in sun protection and bronzing products (SCCNFP/0765/03). This opinion states that the provided data "*do not justify a higher limit than 1 ppm (not to be intentionally added) for furocoumarins in cosmetics*". The SCCNFP also stated *that the opinion should be interpreted in conjunction with the opinion on CMR substances* (SCCNFP/0474/01, final, adopted 25 September 2001).

At the same meeting the SCCNFP also adopted an opinion on furocoumarin Isopimpinellin (SCCNFP/0761/03) stating "*that there is incomplete information on photo-mutagenicity and on*

*photo-clastogenicity of isopimpinellin to enable a safety evaluation in order to provide an update of the "Initial List of Fragrance*"" for entry n° 6.

At the same meeting the SCCNFP also adopted an opinion on the furocoumarin Bergamottin (SCCNFP/0740/03) stating "*Photo-mutagenicity and photo-carcinogenicity are the main effects of concern in relation to the use of furocoumarins in cosmetics. The data submitted by EFFA on bergamottin is not adequate for evaluation of the safety of the substance in relation to photo-mutagenicity and photo-carcinogenicity."*

Submission III was sent to the Commission in April 2005 by EFFA.

## 2. TERMS OF REFERENCE

1. *Is the SCCP of the opinion that the limitation to below 1 ppm of furocoumarins should be extended to all finished cosmetic products and not only cover sun protection and bronzing products?*

2. *Does the SCCP recommend any restrictions with regard to the use of the 11 plants extracts, which in the SCCNFP opinion (SCCNFP/0392/00) are known to contain furocoumarins when they are used as ingredients as such in cosmetics and not as ingredients in fragrances?*

## 3. OPINION

The following documents were submitted to support the terms of references:

* SaitaT, Fujito H and Mori M (2004) Screening of furocoumarin derivatives in citrus fruits by enzyme-linked immunosorbent assay. Biol Pharmacol Bull 27: 974-977.
* Frérot E and Decorzant E (2004) Quantification of total furocoumarins in citrus oils by HPLC coupled with UV, fluorescence and mass detection. J Agr. Food Chem. 52: 6879-6886.
* IFRA/RIFM Furocoumarins Fact Sheet: Furocoumarins in essential oils. (Angelicin, bergamottin, 5-methoxypsoralen, psoralen)

3.1. Screening of furocoumarin derivatives in citrus fruits by enzyme-linked immunosorbent assay. Saita et al (2004) Biol Pharmacol Bull 27: 974-977

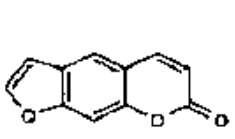
This paper describes an enzyme-linked immunosorbent assay for screening of furocoumarin derivatives as cytochrome P450 3A4 inhibitor in citrus fruits. The antibody used in this assay was produced by immunizing rabbits with 6′,7′-dihydroxybergamottin conjugated with serum albumin. The assay revealed that this antibody could detect both 6′,7′-dihydroxybergamottin and bergamottin, detection limits 800 pg/ml and 4 ng/ml. The specificity testing of the antibody, determined by the cross reactivity with other types of furocoumarin derivatives, revealed 17.2%, 0.28 % and <0.06% (not detectable) cross reaction respectively with bergamottin, 5-methoxy-psoralen and 8-methoxypsoralen for 50% replacement of β-Gal activity. Thus the antibody was

considered to be specific for furocoumarins containing geranyloxy side chain as in bergamottin and 6′,7′-dihydroxybergamottin, the inhibitors of P450 3A4 inhibitor in citrus fruits.

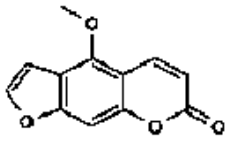
3.2. Quantification of total furocoumarins in citrus oils by HPLC coupled with UV, fluorescence, and mass detection. Frérot and Decorzanr (2004) J Agr. Food Chem. 52: 6879-6886

In this study, an analytical method employing a combination of two high performance liquid chromatography (HPLC) methods and detections by UV diode array, fluorescence and atmospheric pressure chemical ionisation (APCI)-mass spectrometry (MS) was developed for the detection and quantification of 15 furocoumarins (see below). Quantification by UV, fluorescence, or MS was compared in terms of linearity and limit of detection. The method would theoretically allow for the quantification of these 15 furocoumarins at 0.1 ppm level in the sample injected in HPLC. However, some interference was observed when 6 different citrus oils (see below) were analysed employing diode array detection. This problem could be solved by the use of other detection systems, in addition to diode array detection. The authors concluded that the method could be implemented in quality control laboratories. The authors also noted that cold-pressed citrus oils generally contain a high amount of total furocoumarins and would have to be distilled prior to their use in cosmetic products to fall below the permitted level of 1 ppm.

Furocoumarins analysed in the above study are:



*Psoralen*



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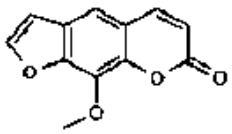
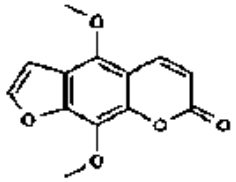
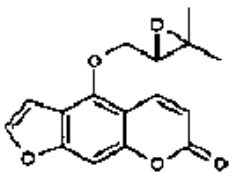


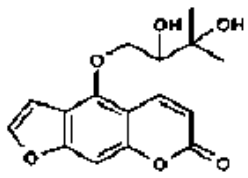
*Xanthotoxin*



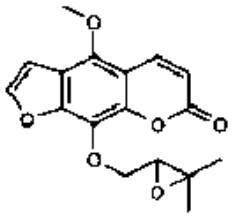
*Isopimpinellin*



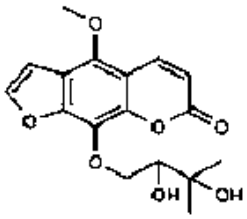
*Oxypeucedanin*



*Oxypeucedanin hydrate*



*Byakangelicol*



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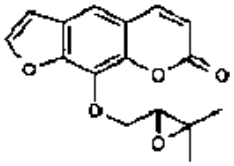
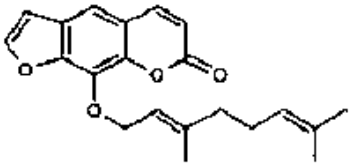
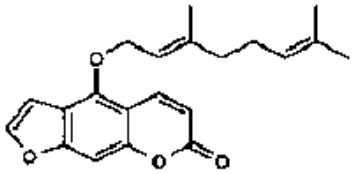


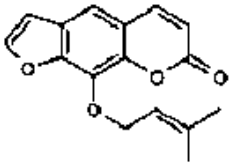
*Heraclenin*



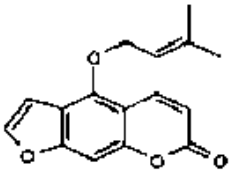
*8-Geranyloxypsoralen*



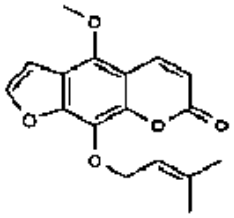
*Bergamottin*



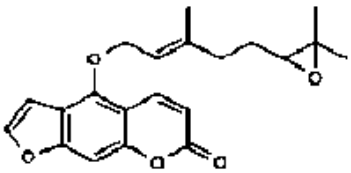
*Imperatorin*



*Isoimperatorin*



*Phellopterin*



*Epoxybergamottin*

Citrus oils analysed in the above study: Bergamot oil, lemon oil, grapefruit oil, mandarin oil, tangerine oil and bitter orange oil

### 3.3. IFRA/RIFM Fact Sheet: Furocoumarins in essential oils, Submission Number 3

The Fact Sheet describes the chemical names, CAS No. EINECS No., molecular formula and molecular weight of some furocoumarins. The EU regulation concerning furocoumarins in cosmetic products is also described in the Fact Sheet. Further, it is stated that the amounts of furocoumarins in plant extracts may vary in a wide range. So far only alkoxyfurocoumarins, having alkyl groups of 11 carbons or less, have been identified, but it is completely plausible that other furocoumarins exist in natural essences, which have so far escaped detection. However, reliable methods are available for only the major furocoumarins (Ferrot and Decorzant, 2004). The results of some analyses of these are described in Table 1. The variations shown in Table 1 clearly indicate that there is no single “marker” furocoumarin that can be used to allow

estimation of total furocoumarin levels. It is also mentioned in the Fact Sheet that "it seems that not all furocoumarins have equivalent biological properties"

## 4. DISCUSSION

The data provided in Submission III has shown that there are many furocoumarin-like substances present in citrus oils. Some of these can be identified and quantified by the newly developed methods. Several furocoumarins have been recognised as phototoxic and SCCNFP has evaluated consumer safety by the use of cosmetic products containing furocoumarins, as already described in the 'Background' to this opinion. The data provided, so far, has not ruled out the photo-toxicity of any of the known furocoumarins. The consumer is exposed to sunlight after application of various types of cosmetic products, not only after the application of bronzing and sun protection products. According to earlier SCCNFP Opinions (SCCNFP/0392/00, SCCNFP/0765/03), the total concentration of furocoumarin-like substances should not exceed 1 ppm in the finished cosmetic product.

In the absence of any additional new data demonstrating the safety of any of the furocoumarin-like substances, the SCCP reemphasise that total concentrations of furocoumarins exceeding 1 ppm in any finished cosmetic product will be of concern with regard to consumer safety.

## 5. CONCLUSION

The data provided so far has not ruled out the photo-toxicity of any furocoumarin. No new data is provided in Submission III to substantiate consumer safety when using cosmetic products containing furocoumarins.

As the consumer is exposed to sunlight after using various types of cosmetic products, not only bronzing or sun protection products, the SCCP reemphasise that total concentrations of furocoumarins exceeding 1 ppm in any finished cosmetic product will be of concern with regard to consumer safety.

The Opinion concerns the content of furocoumarins in cosmetic formulations, irrespective of the source of these substances.

This Opinion should be interpreted together with the opinion on CMR substances (SCCNFP/0474/01, Final).

**IFRA** **RIFM**

## Furocoumarins – Fact Sheet

### Table 1: Summary of furocoumarin analysis in citrus oils

| Essential Oil | FUROCOUMARIN | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | PSORALEN | BERGAPTEN (5-MOP) | XANTHOTOXIN (8-MOP) | ISOPIMPINELLIN | BERGAMOTTIN | OXYPEUCEDANIN | ANGELICIN | EPOXY-BERGAMOTTIN |
| Angelica root oil | up to 112 ppm | up to 78 ppm | not detected | not detected | not detected | ? | 230 ppm | ? |
| Bergamot oil | 0-26 ppm * | up to 2300 ppm (0.23%) | not detected | not detected | up to 2.22% | not detected | not detected | ? |
| Grapefruit oil | not detected | up to 1900 ppm (0.19%) | not detected | < 5 ppm | up to 1100 ppm (0.11%) | not detected | not detected | 1126 ppm |
| Lemon oil, cold pressed | 0-3 ppm * | up to 275 ppm | not detected | 0-18 ppm * | up to 5412 ppm (0.54%) | up to 8224 ppm (0.82%) | not detected | ? |
| Lime oil, cold pressed | < 5 ppm | up to 2200 ppm (0.22%) | < 5 ppm | 2000 (0.2%) | 2.5% | 1200 ppm (0.12%) | not detected | ? |
| Mandarin oil | not detected | 0-3 ppm* | not detected | not detected | 0-10 ppm* | not detected | not detected | ? |
| Orange oil bitter | 70 ppm | 350 ppm (0.035%) | not detected | not detected | 5 ppm | not detected | not detected | 820 ppm[1] |
| Rue oil | 150 ppm | 180 ppm | 320 ppm | 200 ppm | not detected | ? | 430 ppm | ? |
| Tagetes oil | 110 ppm | not detected | not detected | not detected | not detected | ? | not detected | ? |

All values reported were measured by the fragrance industry unless noted. Furocoumarin peaks were identified by comparing the retention time and the UV-spectrum in the sample solution with that of the standard solution.

* Qualitative determination was performed using the retention time of the reference (the amount was too low for a UV-spectrum)

[1] McHale and Sheridan, 1989. The oxygen heterocyclic compounds of citrus peel oils. Proceedings of the 11th International Congress of Essential Oils. Delhi, India, 12-16 November.

## 6. MINORITY OPINION

Not applicable

## 7. REFERENCES

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* Frérot E and Decorzant E (2004) Quantification of Total Furocoumarins in Citrus Oils by HPLC Coupled with UV, Fluorescence, and Mass Detection. J. Agric. Food Chem. 2004, 52, 6879-6886.
* SaitaT, Fujito H and Mori M (2004) Screening of Furanocoumarin Derivatives in Citrus Fruits by Enzyme-Linked Immunosorbent Assay. Biol. Pharm. Bull. 27(7) 974-977 (2004).
* Opinion of the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers concerning an initial list of perfumery materials which must not form part of cosmetic products except subject to the restrictions and conditions laid down. Adopted by the SCCNFP during the 18th Plenary meeting of 25 September 2001. Doc. n° SCCNFP 0392/00
* Opinion of the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers concerning chemical ingredients in cosmetic products classified as carcinogenic, mutagenic or toxic to reproduction according to the chemicals Directive 67/548/EEC. Adopted by the SCCNFP during the 18th Plenary meeting of 25 September 2001. Doc. n° SCCNFP/0474/01
* Opinion of the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers concerning furocoumarins in sun protection and bronzing products. Adopted by the SCCNFP during the 26th plenary meeting of 9 December 2003. Doc. n° SCCNFP/0765/03

## 8. ACKNOWLEDGEMENTS

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| | | |
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# Safety Assessment of Citrus-Derived Peel Oils as Used in Cosmetics

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**Abstract**

The Cosmetic Ingredient Review Expert Panel assessed the safety of 14 citrus-derived peel oil ingredients and concluded that these ingredients are safe for use in cosmetic products when finished products, excluding rinse-off products, do not contain more than 0.0015% (15 ppm) 5-methoxypsoralen, and when formulated to be nonsensitizing and nonirritating. The citrus-derived peel oil ingredients are most frequently reported to function in cosmetics as fragrances and/or skin conditioning agents. The Panel reviewed the available animal and clinical data to determine the safety of these ingredients. Because final product formulations may contain multiple botanicals, each containing the same constituents of concern, formulators are advised to be aware of these constituents and to avoid reaching levels that may be hazardous to consumers. Industry should use good manufacturing practices to limit impurities that could be present in botanical ingredients.

**Keywords**

cosmetics, safety, citrus peel oils

## Introduction

Citrus-derived peel oils are widely used as cosmetic ingredients and are most frequently reported to function in cosmetics as fragrances and/or skin conditioning agents (Table 1). This report assesses the safety of the following 14 citrus-derived peel oils:

*Citrus aurantifolia* (lime) peel oil
*Citrus aurantium amara* (bitter orange) peel oil
*Citrus aurantium currassuviensis* peel oil
*Citrus aurantium dulcis* (orange) peel oil
*Citrus clementina* peel oil
*Citrus grandis* (grapefruit) peel oil
*Citrus iyo* peel oil
*Citrus junos* peel oil
*Citrus limon* (lemon) peel oil
*Citrus medica vulgaris* peel oil
*Citrus nobilis* (mandarin orange) peel oil
*Citrus reticulata* (tangerine) peel oil
*Citrus tachibana/reticulata* peel oil
*Citrus tangerina* (tangerine) peel oil

The citrus ingredients in this assessment are found in foods, and daily exposure from food use would result in much larger systemic exposures than those from use in cosmetic products. Additionally, essential oils, oleoresins (solvent-free), and natural extracts (including distillates) derived from some citrus fruits are generally recognized as safe (GRAS) for their intended use in foods for human and animal consumption according to the US Food and Drug Administration (FDA). Volatile oils of limes, lemons, grapefruits, bitter oranges, oranges, and tangerines are described as flavoring agents in the US Pharmacopeia (USP) Food Chemicals Codex.[1] Thus, the systemic toxicity potential of citrus-derived peel oils via oral exposure is not addressed further in this report. The primary focus of this safety assessment of these citrus ingredients as used in cosmetics is on the potential for irritation and sensitization from dermal exposure.

The Cosmetic Ingredient Review (CIR) does not review ingredients that function only as fragrance ingredients because, as fragrances, the safety of these ingredients is evaluated by the Research Institute for Fragrance Materials (RIFM). Three of the citrus-derived peel oils in this report function only as

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**Table 1.** Definitions and Functions of Citrus-Derived Ingredients.

| Ingredient | Definition[2] | Function[2] |
|---|---|---|
| *Citrus aurantifolia* (lime) peel oil | *C aurantifolia* (lime) peel oil is the volatile oil obtained from the peel of *C aurantifolia* | Fragrance ingredients |
| *Citrus aurantium* amara (bitter orange) peel oil<br>CAS No. 68916-04-1 | *C aurantium* amara (bitter orange) peel oil is the volatile oil obtained from the peel of *C aurantium amara* | Fragrance ingredients; skin conditioning agents-miscellaneous |
| *Citrus aurantium currassuviensis* peel oil | *C aurantium currassuviensis* peel oil is the volatile oil derived from the peel of the laraha orange, *C aurantium currassuviensis* | Fragrance ingredients |
| *Citrus aurantium dulcis* (orange) peel oil<br>CAS No. 8008-57-9 | *C aurantium dulcis* (orange) peel oil is the volatile oil obtained by expression from the peel of *Citrus sinensis* | Fragrance ingredients; skin conditioning agents-miscellaneous |
| *Citrus clementina* peel oil | *C clementina* peel oil is the volatile oil obtained from the peel of *C clementina* | Fragrance ingredients; skin conditioning agents-miscellaneous |
| *Citrus grandis* (grapefruit) peel oil<br>CAS No. 8016-20-4 | *C grandis* (grapefruit) peel oil is the volatile oil obtained from the peel of the grapefruit, *C grandis* | Fragrance ingredients; skin conditioning agents-miscellaneous |
| *Citrus iyo* peel oil | *C iyo* peel oil is the volatile oil obtained from the peel of *C iyo* | Skin conditioning agents-emollient |
| *Citrus junos* peel oil | *C junos* peel oil is the volatile oil obtained from the peel of *C junos* | Cosmetic astringents |
| *Citrus limon* (lemon) peel oil<br>CAS Nos. 8008-56-8; 8020-19-7; 84929-31-7; 85085-28-5 | *C limon* (lemon) peel oil is the volatile oil obtained from the peel of *C limon* | Fragrance ingredients; skin conditioning agents-miscellaneous |
| *Citrus medica vulgaris* peel oil | *C medica vulgaris* peel oil is the volatile oil obtained from the peel of *C medica vulgaris* | Fragrance ingredients |
| *Citrus nobilis* (mandarin orange) peel oil<br>CAS No. 8008-31-9; 84696-35-5 | *C nobilis* (mandarin orange) peel oil is the oil obtained from the peel of the mandarin orange, *C nobilis* | Fragrance ingredients; skin conditioning agents-miscellaneous |
| *Citrus reticulata* (tangerine) peel oil<br>CAS No. 8008-31-9 | *C reticulata* (tangerine) peel oil is the volatile oil obtained from the peel of *C reticulata* | Deodorant agents; flavoring agents; fragrance ingredients |
| *Citrus tachibana/reticulata* peel oil | *C tachibana/reticulata* peel oil is the volatile oil obtained from the peel of the hybrid of *C tachibana* and *C reticulata* | Skin conditioning agents-emollient |
| *Citrus tangerina* (tangerine) peel oil | *C tangerina* (tangerine) peel oil is the volatile oil obtained from the peel of *C tangerina* | Fragrance ingredients; skin conditioning agents-miscellaneous |

**Table 2.** Citrus Ingredients That Potentially Function Solely as Fragrance Ingredients.

| |
|---|
| *Citrus aurantifolia* (lime) peel oil |
| *Citrus aurantium currassuviensis* peel oil |
| *Citrus medica vulgaris* peel oil |

fragrance ingredients, according to the *International Cosmetic Ingredient Dictionary and Handbook* (see Table 2).[2] However, RIFM has not confirmed that these 3 ingredients function only as fragrances and therefore within its purview; thus, CIR is reviewing the safety of these ingredients.

Botanicals such as those derived from citrus contain hundreds of constituents, some of which have the potential to cause toxic effects. For example, bergapten (also known as 5-methoxypsoralenor [5-MOP]) is a naturally occurring phototoxic furanocoumarin (psoralen) in citrus peel oils. In this assessment, CIR is reviewing information available to evaluate the potential toxicity of each of the citrus-derived peel oils as a whole, complex substance. Except for specific constituents of concern, CIR is not reviewing information that may be available to assess the potential toxicity of the individual constituents of which the citrus-derived ingredients are composed. Cosmetic Ingredient Review requested information on the concentrations (including ranges, means, upper 95%confidence limits, detection limits, etc) of individual constituents in citrus-derived peel oils used in cosmetics to facilitate the safety assessment of these ingredients. Such information on constituents that have been identified as constituents of concern by the Panel in previous safety assessments, or by other recognized scientific expert review bodies, is especially important.

Some toxicological data on lemon oil and sweet orange oil (synonyms: lemon, ext. and orange, sweet, extract, respectively) reviewed in this safety assessment were obtained from robust summaries of data submitted to the European Chemical Agency (ECHA) by companies as part of the REACH chemical registration process. These data are available on the ECHA website.[3,4]

Note that, in many of the published studies included in this assessment, the information provided is not sufficient to determine how well the substance being tested represents the cosmetic ingredient. In this safety assessment, if a substance tested in a study is not clearly a cosmetic ingredient, because of lack of information on the genus and species from which the substance was derived and/or the method of extraction used, the test substance will be referred to by a common name (eg, lime oil). If the substance is clearly a cosmetic ingredient, the International Nomenclature of Cosmetic Ingredients (INCI) name

will be used (eg, "*C aurantifolia* [lime] peel oil"). In some instances, the part of the plant from which the oil was expressed is not known but, based on the method of manufacture, the oil could have been expressed from the peels of the citrus fruit. Additionally, some inconsistencies were noted in both taxonomic and INCI naming conventions. For example, this report includes the sweet orange ingredient described as *C aurantium dulcis* (orange) peel oil in the *International Cosmetic Ingredient Dictionary and Handbook* (*Dictionary*).[2] In contrast, most of the published literature and the FDA refer to this ingredient as *Citrus sinensis* (sweet orange) peel oil. Another example of a naming inconsistency is *C grandis* (grapefruit) peel oil; *C grandis* is generally considered a name for a pummelo and is more commonly called *Citrus maxima*. The INCI Committee of the Personal Care Products Council (Council) is working to correct some of these errors.

## Chemistry

### Definition and General Characterization

The definitions and functions of the citrus-derived peel oils included in this report are provided in Table 1. In some cases, the definition provides insight on the method(s) of manufacture. It should be noted that essential oils are hydrophobic, liquid, and volatile aroma compounds from plants. These are typically mixtures of small molecules, but their chemical structures can vary widely. The volatile nature of essential oils makes them likely to be useful as fragrances but does not preclude other functions for them in cosmetics.

There are numerous *Citrus* varieties distributed throughout the world; most citrus fruits are grown in the temperate and tropical zones in both the northern and southern hemispheres.[5] Citrus can be propagated and new varieties can be produced by cultivating asexual nuclear or chance seedlings or by crossing or mutation.

### Physical and Chemical Properties

Physical and chemical properties of the citrus-derived peel oils are provided in Table 3.

### Method of Manufacturing

A suitable antioxidant can be added to citrus oil during preparation.[1]

Bitter orange oil and orange oil are obtained using a cold-pressed extraction method from the fresh peel of the fruit of *Citrus aurantium* and *C sinensis*, respectively.[1] *Citrus grandis* oil[6] and *C junos* oil[7] are also extracted using a cold-press method. Tangerine oil (cold-pressed) is obtained from the peels of the ripe fruit of the Dancy tangerine, *C nobilis* or *C reticulata*, and from some other closely related varieties.[1]

Lemon oil and lime oil, expressed, are produced by pressing the outer rind of the ripe fruit manually or by machine.[8] More economical processes involve an integrated juice-oil procedure. These oils can also be produced by distillation of expressed oils or direct distillation of fruit; distilling (rectifying) removes terpenes. Steam distillation removes nonvolatile furocoumarins.

*Citrus iyo* oil is produced by removing the peel from the fruit, homogenization of the peel with distilled water, and lyophilization.[9] The oil is purified using vacuum distillation.

Mandarin peel oil, expressed (identified in the literature as *C reticulata*), is prepared by expression of the peels of the ripe fruit of the mandarin orange.[10]

### Constituents/Composition

The citrus-derived peel oils are complex botanicals composed of numerous constituents; there is great variation among citrus species and cultivars because of frequent bud mutations, interspecific and intergeneric hybridization, and apomixis (ie, 1 or more of several types of asexual reproduction).[11] The composition of citrus oils will vary based on the location where the plant is grown, the maturity of the plant, and storage conditions.[5] The method of extraction will also affect the composition.

Citrus oils contain large amounts of monoterpene hydrocarbons. Limonene is the constituent present in the greatest amount, often comprising greater than 90% of the oil, and the amount present can vary within the oil; for example, limonene is reported to compose 38.1% to 95.8% *C limon* (lemon) peel essential oils, cold-pressed.[12,13] Citrus oils also contain sesquiterpene hydrocarbons, which are responsible for the characteristic flavors of these oils.[12] Table 4 lists the chemical compositions of cold-pressed,[6,7,11-16] hydrodistilled,[17-21] lyophilization/vacuum distillation,[9] and steam-distilled[22] peel oils sorted by citrus plant species; Table 5 presents the typical levels of 5-MOP found in some of the oils; and Table 6 provides the levels of major coumarins and furocoumarins in lemon and lime oil. Table 7 lists citrus constituents that are established contact allergens, according the European Commission's Scientific Committee on Consumer Safety.

## Use

### Cosmetic

Table 8 presents the current product-formulation use data for citrus-derived peel oils. These ingredients are most frequently reported to function as fragrances and/or skin conditioning agents-miscellaneous.[2]

According to information supplied to the FDA by industry as part of the Voluntary Cosmetic Registration Program (VCRP), *C limon* (lemon) peel oil has the most reported uses in cosmetic and personal care products, with a total of 490; more than half of the uses are in leave-on skin care preparations.[23] *Citrus aurantium dulcis* (orange) peel oil (reported as *C sinensis* [sweet orange] peel oil by the VCRP) has the second greatest number of overall uses reported, with a total of 289; about half of those uses are in leave-on skin care preparations.

In a use concentration survey conducted by the Council, *C limon* (lemon) peel oil had a maximum use concentration range

**Table 3.** Physical and Chemical Properties of Citrus-Derived Peel Oils.

| Property | Description | Reference |
|---|---|---|
| *Citrus aurantifolia* (lime) oil | | |
| Color | Colorless to greenish yellow | 8 |
| Odor | Fresh citrus, intense | 8 |
| Optical rotation | +34° to +47° | 8 |
| Solubility | Insoluble in water, soluble in ethanol and propylene glycol | 8 |
| Refractive index | 1.4477-1.4745 | 8 |
| Specific gravity | 0.855-0.863 | 8 |
| *C aurantifolia* or *Citrus latifolia* (lime) oil (distilled) | | |
| Color | Colorless to greenish yellow | 1 |
| Odor | Mild citrus, floral | 1 |
| Optical rotation/angular rotation | +34° to +47° | 1 |
| Solubility | Soluble in most fixed oils and mineral oil; insoluble in glycerin and propylene glycol | 1 |
| Solubility in alcohol | 1-mL sample dissolves in 5 mL of 90% alcohol | 1 |
| Aldehydes | Between 0.5% and 2.5% of aldehydes, calculated as citral | 1 |
| Refractive index | 1.474-1.477 at 20°C | 1 |
| Specific gravity | 0.855-0.863 | 1 |
| *C aurantifolia* (lime) oil (cold-pressed) | | |
| Color | Yellow to brown-green to green liquid | 1 |
| Odor | Fresh lime peel | 1 |
| Optical rotation/angular rotation | Mexican type: +35° to +41°; Tahitian type: +38° to +53° | 1 |
| Solubility | Soluble in most fixed oils and mineral oil; insoluble in glycerin and propylene glycol | 1 |
| Aldehydes | Mexican type: no less than 4.5% and no more than 8.5% of aldehydes, calculated as citral; Tahitian type: no less than 3.2% and no more than 7.5% of aldehydes, calculated as citral | 1 |
| Refractive index | Mexican type: 1.482-1.486; Tahitian type: 1.476-1.486 | 1 |
| Residue of evaporation | Mexican type: 10.0%-14.5%; Tahitian type: 5.0%-12.0% | 1 |
| Specific gravity | Mexican type: 0.872-0.881; Tahitian type: 0.858-0.876 | 1 |
| UV absorbance | Max. at 315 nm; Mexican type: no less than 0.45; Tahitian type: no less than 0.24 | 1 |
| *Citrus aurantium* (bitter orange) oil (cold-pressed) | | |
| Color | Pale yellow or yellow-brown liquid | 1 |
| Odor | Characteristic aromatic odor of the Seville orange | 1 |
| Optical rotation/angular rotation | +88° to +98° | 1 |
| Solubility | Miscible with absolute alcohol and with an equal volume of glacial acetic acid; soluble in fixed oils and mineral oil; slightly soluble in propylene glycol; relatively insoluble in glycerin | 1 |
| Aldehydes | No less than 0.5% and no more than 1.0% of aldehydes, calculated as decyl aldehyde | 1 |
| Refractive index | 1.472-1.476 at 20°C | 1 |
| Residue of evaporation | 2.0%-5.0% | 1 |
| Specific gravity | 0.845-0.851 | 1 |
| *Citrus clementina* | | |
| Color | Orange to brownish liquid | 43 |
| Odor | Characteristic | 43 |
| Optical rotation | +89 to +97° (15°C) | 43 |
| Solubility | Insoluble in water; soluble in ethyl alcohol | 43 |
| Refractive index | 1.5640-1.4820 (20°C) | 43 |
| Specific gravity | 0.840-0.875 (15°C) | 43 |
| *Citrus limon* (lemon) oil | | |
| Color | Pale to deep yellow or greenish yellow | 8 |
| Odor | Fresh citrus, intense | 8 |
| Optical rotation | +57 to +65.6 | 8 |
| Solubility | Insoluble in water, soluble in ethanol and propylene glycol | 8 |
| Refractive index | 1.474-1.467 | 8 |
| Specific gravity | 0.849-0.855 | 8 |
| *C limon* (lemon) oil (distilled) | | |
| Color | Colorless to pale yellow liquid | 1 |
| Odor | Fresh lemon peel | 1 |
| Optical rotation/angular rotation | +55° to +75° | 1 |
| Solubility | Soluble in most fixed oil, mineral oil, and alcohol (with haze); insoluble in glycerin and propylene glycol | 1 |
| Solubility in alcohol | 1-mL sample dissolves in 5 mL of 90% alcohol | 1 |

(continued)

**Table 3.** (continued)

| Property | Description | Reference |
|---|---|---|
| Aldehydes | Between 1.0% and 3.5% of aldehydes, calculated as citral | 1 |
| Refractive index | 1.470-1.475 at 20°C | 1 |
| Specific gravity | 0.842-0.856 | 1 |
| UV absorbance | Maximum at 315 nm, no less than 0.01 | 1 |
| *C limon* (lemon) oil (cold-pressed) | | |
| Color | Pale to deep yellow or green-yellow | 1 |
| Odor | Fresh lemon peel | 1 |
| Optical rotation/angular rotation | California and Italian types: +57° to +65.6°; desert type: +67° to +78° | 1 |
| Solubility | Miscible with dehydrated alcohol and glacial acetic acid | 1 |
| Solubility in alcohol | 1 mL of sample dissolves in 3 mL of 95% alcohol, slight haze possible | 1 |
| Aldehydes | Desert type: no less than 1.7% of aldehydes, calculated as citral; California type: no less than 2.2% and no more than 3.8% of aldehydes, calculated as citral; Italian type: no less than 3.0% and no more than 5.5% of aldehydes, calculated as citral | 1 |
| Refractive index | 1.473-1.476 at 20°C | 1 |
| Residue of evaporation | Between 5.0% and 14.5% | 1 |
| Specific gravity | Desert type: 0.846-0.851; California and Italian types: 0.849-0.855 | 1 |
| UV absorbance | Maximum at 315 nm; desert and California types: no less than 0.2; Italian type: no less than 0.49 | 1 |
| *Citrus nobilis or Citrus reticulata* (tangerine) oil (cold-pressed) | | |
| Color | Red-orange to brown-orange | 1 |
| Odor | Pleasant, orange | 1 |
| Optical rotation/angular rotation | +88° to +96° | 1 |
| Solubility | Soluble in most fixed oils and mineral oil; slightly soluble in propylene glycol; relatively insoluble in glycerin | 1 |
| Aldehydes | 0.8% to 1.9% of aldehydes, calculated as decyl aldehyde | 1 |
| Refractive index | 1.473-1.476 at 20°C | 1 |
| Residue of evaporation | 2.3%-5.8% | 1 |
| Specific gravity | 0.844-0.854 | 1 |
| *Citrus reticulata* peel oil | | |
| Physical state and appearance | Clear, mobile, dark orange to reddish-orange or Brownish-orange liquid | 10 |
| Odor | Orange-like | 10 |
| Optical rotation | +63° to +78° | 10 |
| Refractive index | 1.4730-1.4770 (20°C) | 10 |
| Specific gravity | 0.847-0.853 (25/25°C) | 10 |
| *Citrus sinensis* (orange) oil (distilled) | | |
| Color | Colorless to pale yellow liquid | 1 |
| Odor | Mild citrus floral | 1 |
| Optical rotation/angular rotation | +94° to +99° | 1 |
| Solubility | Soluble in most fixed oil, mineral oil, and alcohol (with haze); insoluble in glycerin and propylene glycol | 1 |
| Solubility in alcohol | 1-mL sample dissolves in 5 mL of 90% alcohol | 1 |
| Refractive index | 1.471-1.474 at 20°C | 1 |
| Specific gravity | 0.840-0.844 | 1 |
| UV absorbance | Maximum at 330 nm, no less than 0.01 | 1 |
| *Citrus sinensis* (orange) oil (cold-pressed) | | |
| Color | Intensely yellow, orange, or deep orange liquid | 1 |
| Odor | Characteristic of fresh, sweet orange peel | 1 |
| Optical rotation/angular rotation | +94° to +99° | 1 |
| Solubility | Miscible with dehydrated alcohol and carbon disulfide; soluble in glacial acetic acid | 1 |
| Aldehydes | No less than 1.2% and no more than 2.5% of aldehydes, calculated as decyl aldehyde | 1 |
| Refractive index | 1.472-1.474 at 20°C | 1 |
| Specific gravity | 0.842-0.846 | 1 |
| UV absorbance | Maximum at 330 nm; California type: no less than 0.130; Florida type: no less than 0.240 | 1 |

Abbreviation: UV, ultraviolet.

of 0.0001% to 0.5%, with 0.5% reported in "other" skin care preparations.[24] *Citrus aurantium dulcis* (orange) peel oil had a maximum use concentration range of 0.00002% to 29%, with 29% reported in noncoloring hair conditioners.

In some cases, reports of uses were received from the VCRP, but no concentration of use data were provided. For example, *C junos* peel oil is reported to be used in 6 formulations, but no use concentration data were available. In other

**Table 4.** Chemical Composition (%) of Citrus Peel Oils.[a]

| | *Citrus aurantifolia* (lime) peel oil | | *Citrus aurantium amara* (bitter orange) essential oil | | | | *Citrus aurantium dulcis* (orange) essential oil | | *Citrus clementina* Peel essential oil | | *Citrus grandis* (grapefruit) peel essential oil | | *Citrus iyo* peel essential oil | | *Citrus junos* peel essential oil | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cold-pressed[13] | | Cold-pressed[14] | | Hydrodistilled[18,20] | | Cold-pressed[14] | | Hydrodistillation[17] | | Cold-pressed[6] | | Lyophilization/vacuum distillation[9] | | Cold-pressed[7] or not specified[16] | |
| | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max |
| Essential oil content (%) | | | | | 0.12[18]-1.67[20] | | | | | | | 0.12[6] | | 1.5[9] | | |
| Constituent | | | | | | | | | | | | | | | | |
| Limonene | 39.9 | 94.4 | | | 67.9 | 94.67 | | | 93.77 | 96.19 | | 94.8 | 79 | 89 | 63.1 | 77.44 |
| (E)-α-Bergamotene | | | | | | | | | | | | | | | Trace | |
| (E)-β-farnesene | Trace | 0.1 | | | | | | | | | | | | | Trace | |
| (E)-β-ocimene | Trace | 0.5 | | | 0.02 | 0.5 | | | | | | | | | ND | Trace |
| (E)-decenal | | | | | | | | | | | | | | | ND | Trace |
| (E)-carvone | | | | | | | | | | | | 0.005-0.05 | | | | |
| (E)-caryophyllene | 0.1 | 0.5 | | | | | | | | | | | | | | |
| (E)-caryophyllene oxide | | | | | | | | | | | | Trace | | | | |
| (E)-linalool oxide | | | | | | | | | | | | | | | ND | Trace |
| (E)-nerolidol | | | | | | | | | | | | | | | Trace | |
| (E)-p-mentha-2,8-dienol | | | | | | | | | | | | 0.1 | | | | |
| (E)-p-2,8-menthadien-1-ol | | | | | | | | | | | | Trace | | | | |
| (E, E)-α-farnesene | 0.3 | 1.9 | | | | | | | | | | 0.1 | | | | |
| (E, E)-farnesyl acetate | | | | | | | | | | | | | | | ND | Trace |
| (E, Z)-2,6-nonadien-1-ol | | | | | | | | | | | ND | | | | | |
| (Z)-β-ocimene | Trace | 0.7 | | | | | | | | | | | | | Trace | |
| Z-carveol | | | | | | | | | | | | 0.005-0.05 | | | | |
| (Z)-carvone | | | | | | | | | | | | 0.1 | | | | |
| (Z)-β-farnesene | | | | | | | | | | | | | | | 0.9 | 1.3 |
| (Z)-limonene oxide | | | | | | | | | | | | | | | ND | Trace |
| (Z)-piperitol | | | | | | | | | | | | 0.005-0.05 | | | | |
| 1-Octanol | | | | | | | | | | | | | | | | |
| 1,3,8-p-Menthatriene | | | | | | | | | | | | | | | Trace | |
| 1,8-Cineole | 0.5 | 0.6 | | | Trace | 14.72 | | | | | | | | | | |
| 2Z.6E-farnesol | | | | | Trace | | | | | | | | | | | |
| 3-Carene | Trace | 0.3 | | | | | | | | | | | | | | |
| 3,3',4',5,6,7-Heptamethoxyflavone | | | X | | | | X | | | | | | | | | |
| 3,3',4',5,6,7-Hexamethoxyflavone | | | | | | | X | | | | | | | | | |
| 3',4',5,6,7,8-Hexamethoxyflavone | | | X | | | | | | | | | | | | | |
| 3',4',5,6,7-Pentamethoxyflavone | | | | | | | X | | | | | | | | | |
| 4',5,6,7-Tetramethoxyflavone | | | X | | | | X | | | | | | | | | |
| 4',5,6,7,8-Pentamethoxyflavone | | | X | | | | X | | | | | | | | | |
| 5-Methoxypsoralen | | | X | | | | | | | | | | | | | |
| (5-(6',7'-Dihydroxy-geranyloxy)psolaren) | | | X | | | | | | | | | | | | | |
| (5-(6',7'-Epoxy-geranyloxy)psolaren) | | | X | | | | | | | | | | | | | |
| 5,7-Dimethoxycoumarin | | | X | | | | | | | | | | | | | |
| 6-Methylhept-5-en-2-one | | Trace | | | | | | | | | | | | | | |
| 7-Methoxy-8(2',3'-dihydroxy)isopentyloxycoumarin | | | X | | | | | | | | | | | | | |
| 7-Methyoxy-8(2',3'-epoxy)isopentenylcoumarin | | | X | | | | | | | | | | | | | |
| 7-Methyoxy-8-isopentenylcoumarin | | | X | | | | | | | | | | | | | |
| 7-Methoxy-8-(2'-one-isopentyl)coumarin) | | | X | | | | | | | | | | | | | |
| 30 carvacrol | | | | | Trace | 0.69 | | | | | | | | | | |
| Allo-ocimene | | Trace | | | | | | | | | | | | | | |
| Aromadendrene | | | | | | | | | | | | | | | ND | Trace |
| Bicycloelemene | | | | | | | | | | | | | | | Trace | |
| Bicyclogermacrene | Trace | 0.4 | | | | | | | | | | | | | 0.7 | 2.0 |
| Borneol | | | | | 0.08 | 0.16 | | | | | | | | | | |

*(continued)*

**Table 4.** (continued)

| | *Citrus aurantifolia* (lime) peel oil | | *Citrus aurantium amara* (bitter orange) essential oil | | | | *Citrus aurantium dulcis* (orange) essential oil | | *Citrus clementina* Peel essential oil | | *Citrus grandis* (grapefruit) peel essential oil | | *Citrus iyo* peel essential oil | | *Citrus junos* peel essential oil | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cold-pressed[13] | | Cold-pressed[14] | | Hydrodistilled[18,20] | | Cold-pressed[14] | | Hydrodistillation[17] | | Cold-pressed[6] | | Lyophilization/vacuum distillation[9] | | Cold-pressed[7] or not specified[16] | |
| | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max |
| Bornyl acetate | Trace | Trace | | | Trace | | | | | | | | | | | |
| Camphene | Trace | 0.2 | | | Trace | 0.24 | | | | | | | | | Trace | |
| Camphor | | | | | 0.17 | 6.37 | | | | | | | | | | |
| Caryophyllene | | | | | | | | | | | | | | | ND | Trace |
| Caryophyllene oxide | Trace | 0.1 | | | 0.08 | 0.21 | | | | | | Trace | | | | |
| Cedrol | | | | | | | | | | | Trace | | | | | |
| *cis*-Dihydrocarvone | | | | | | | 0.03 | 0.28 | | | | | | | | |
| *cis*-Limonene-1,2-oxide | Trace | 0.1 | | | | | | | | | | | | | | |
| *cis*- Linalool oxide THF | | Trace | | | Trace | 3.4 | | | | | | | | | | |
| *cis*-β-Ocimene | | | | | | 0.30 | | | | | | | | | | |
| *cis*-Sabinene hydrate | | | | | Trace | 0.18 | | | | | | | | | Trace | 0.1 |
| Citronellal | Trace | 1.6 | | | | | | | 0.03 | 0.08 | | 0.1 | | | | |
| Citronellol | Trace | 0.6 | | | | | Trace | 0.07 | | | | | | | ND | Trace |
| Citronellyl acetate | 0.1 | 0.2 | | | | | | | | | | | | | | |
| Cumin aldehyde | | | | | | | | | | | | 0.005-0.05 | | | | |
| Decanal | Trace | 0.3 | | | | 0.16 | | | | | | 0.3 | | | Trace | |
| Dihydrocarveol | | | | | | | | | | | Trace | | | | | |
| Dodecanol | | Trace | | | | | | | | | | 0.1 | | | ND | Trace |
| Elemol | | | | | | | | | | | Trace | | | | Trace | |
| Eugenol | | | | | | | | | | | | | | | ND | Trace |
| Geranial | 0.5 | 6.1 | | | | | | | | | | 0.1 | | | | |
| Geraniol | Trace | 0.3 | | | | | | | | | | | | | | |
| Geranyl acetate | 0.1 | 2.4 | | | Trace | 0.11 | | | | | | | | | | |
| Geranyl propionate | | | | | | | | | | | Trace | | | | | |
| Germacrene B | | | | | | | | | | | | | | | 0.1 | 0.2 |
| Germacrene D | Trace | 1.0 | | | 0.04 | 0.25 | | | | | | | | | 0.3 | 0.4 |
| Germacrene D-4-ol | | | | | | | | | | | | | | | 0.3 | 0.4 |
| Globulol | | 0.1 | | | | | | | | | | | | | | |
| Guaiene | | | | | | | | | | | | | | | ND | Trace |
| Heptyl acetate | | | | | | | | | | | | 0.3 | | | | |
| Isothyujol | | | | | | | | | | | | 0.005-0.05 | | | | |
| Limonen-10-ol | | | | | | | | | | | Trace | | | | | |
| Linalool | 0.1 | 1.4 | | | Trace | 0.76 | | | 0.03 | 0.08 | | 0.1 | 1.3 | 7.3 | 1.6 | 2.8 |
| Linalool oxide | | | | | | | | | | | | | | | | |
| Linalyl acetate | Trace | Trace | | | 0.01 | 0.18 | | | | | | 0.1 | | | | |
| Methyl-N-methyl anthranilate | | | | | | | | | | | | 0.005-0.05 | | | | |
| Myrcene | 0.8 | 2.0 | | | 0.07 | 2.0 | | | 0.73 | 0.78 | | | X | | 0.4 | 3.2 |
| Neral | Trace | 3.1 | | | | | | | | | Trace | | | | | |
| Nerol | Trace | 0.1 | | | 0.03 | 0.12 | | | | | Trace | | | | ND | Trace |
| Nerolidyl acetate | | | | | | | | | | | Trace | | | | | |
| Neryl acetate | Trace | 2.0 | | | | 0.10 | | | | | | | | | | |
| Nonanal | Trace | 0.1 | | | Trace | 0.18 | | | | | | 0.005-0.05 | | | Trace | |
| Nonanoic acid | | | | | | | | | | | | Trace | | | | |
| Nootkatone | | | | | | | | | | | | 0.1. | | | | |
| Octanal | Trace | 0.1 | | | 0.02 | 1.59 | | | | | | 0.4 | | | Trace | |
| Octyl acetate | Trace | Trace | | | | | | | | | | | | | | |
| p-Cymene | Trace | 5.3 | | | Trace | 0.09 | | | | | | | | | 0.4 | 0.6 |
| p-Cymenene | | 0.1 | | | | | | | | | | | | | | |
| p-Mentha-1-en-9-ol | | | | | | | | | | | Trace | | | | | |

*(continued)*

**Table 4.** (continued)

| | *Citrus aurantifolia* (lime) peel oil | | *Citrus aurantium amara* (bitter orange) essential oil | | | | *Citrus aurantium dulcis* (orange) essential oil | | *Citrus clementina* Peel essential oil | | *Citrus grandis* (grapefruit) peel essential oil | | *Citrus iyo* peel essential oil | | *Citrus junos* peel essential oil | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cold-pressed[13] | | Cold-pressed[14] | | Hydrodistilled[18,20] | | Cold-pressed[14] | | Hydrodistillation[17] | | Cold-pressed[6] | | Lyophilization/vacuum distillation[9] | | Cold-pressed[7] or not specified[16] | |
| | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max |
| Perillaldehyde | | | | | | | | | | | Trace | | | | Trace | |
| Perillyl alcohol | | | | | | | | | | | | | | | Trace | |
| Perillyl aldehyde | | | | | | | | | | | | 0.005-0.05 | | | | |
| Piperitenone | | Trace | | | | | | | | | | | | | Trace | |
| Pseudolimonene | | | | | | | | | | | | | | | Trace | |
| Sabina ketone | | | | | | | | | | | | 0.1 | | | | |
| Sabinene | 0.1 | 19.6 | | | Trace | 0.200.18 | | | | | | 0.4 | | | 0.4 | 0.5 |
| Santal-10-en-2-ol | Trace | Trace | | | | | | | | | | | | | | |
| Spathulenol | | | | | Trace | 0.21 | | | | | | | | | ND | Trace |
| Terpinen-4-ol | Trace | 1.7 | | | 0.01 | 0.54 | | | | | | | | | Trace | 0.1 |
| Terpinolene | Trace | 1.1 | | | 0.02 | 0.22 | | | | | ND | | | | 0.6 | 0.7 |
| Terpinyl acetate | Trace | Trace | | | | | | | | | | | | | | |
| Tetradecane | | | | | | | | | | | | | | | ND | Trace |
| Tetradecenal | | | | | | | | | | | Trace | | | | | |
| Thymol | | | | | | | | | | | | | | | 0.2 | 0.3 |
| Thymyl methyl oxide | 1.2 | 1.6 | | | | | | | | | | | | | | |
| *trans*-α-Bergamotene | 0.2 | 1.6 | | | | | | | | | | | | | | |
| *trans*-Limonene-1,2-oxide | Trace | 0.2 | | | | | | | | | | | | | | |
| *trans*-Sabinene hydrate | Trace | 0.1 | | | | | | | | | | | | | Trace | 0.1 |
| Tri-Cyclen | | | | | 0 | 0.75 | | | | | | | | | | |
| Undecanal | Trace | 0.1 | | | | | | | | | | | | | | |
| Valencene | | | | | 0.04 | 0.2 | | | | | | | | | | |
| (−)-α-Copaene | | | | | | | | | | | | | | | Trace | 0.1 |
| α-Cadinene | | | | | | | | | | | | | | | | 0.1 |
| α-Cadinol | | | | | | | | | | | | | | | ND | Trace |
| α-Cedrene | | | | | | | | | | | | Trace | | | | |
| α-Copaene | | | | | | | | | | | | 0.005-0.05 | | | | |
| α-Cubebene | | | | | | | | | | | | 0.1 | | | Trace | |
| α-Farnesene | | | | | | | | | | | | | | | ND | Trace |
| α-Humulene | Trace | 0.2 | | | 0.03 | 0.13 | | | | | | | | | | 0.1 |
| α-Muurolene | | | | | | | | | | | | | | | Trace | |
| α-p-Dimethyl styrene | | | | | | | | | | | | | | | Trace | |
| α-Phellandrene | Trace | 0.1 | | | | | | | | | | | | | 0.7 | 0.9 |
| α-Pinene | 0.2 | 2.7 | | | 0.03 | 0.53 | | | 0.15 | 0.31 | | 0.5 | | | 2.0 | 2.7 |
| α-Sinensal | | | | | | | | | | | | | | | | |
| α-Terpinene | Trace | 0.4 | | | 0.91 | 1.66 | | | | | | 1.8 | X | | | 0.3 |
| α-Terpineol | Trace | 4.7 | | | 0.07 | 0.35 | | | 0.04 | 0.09 | | 0.1 | | | 0.2 | 0.3 |
| α-Terpinyl acetate | | | | | Trace | 1.4 | | | | | | | | | | |
| α-Thujene | Trace | 0.7 | | | 0.14 | 0.44 | | | | | ND | | | | | |
| α-Thujone | | | | | Trace | 0.35 | | | | | | | | | | |
| α-ylangene | | | | | | | | | | | | | | | ND | Trace |
| β-Bisabolene | 0.3 | 2.4 | | | | | | | | | | | | | | |
| β-Caryophyllene | | | | | | | | | | | | 0.1 | | | | 0.3 |
| β-Cubebene | | | | | | | | | | | | | | | Trace | 0.1 |
| β-Eleme | Trace | 0.8 | | | | | | | | | | | | | | 0.1 |
| β-Eudesmol | | | | | | | | | | | | | | | Trace | |
| β-Ionone | | | | | | | | | | | | | | | ND | Trace |
| β-Phellandrene | 0.2 | 0.8 | | | | | | | | | | | | | 2.16 | 5.4 |
| β-Pinene | Trace | 19.2 | | | 0.07 | 0.62 | | | 0.06 | 0.36 | | 0.005-0.05 | | | 0.9 | 1.1 |

*(continued)*

**Table 4.** (continued)

| | *Citrus aurantifolia* (lime) peel oil | | *Citrus aurantium amara* (bitter orange) essential oil | | | | *Citrus aurantium dulcis* (orange) essential oil | | *Citrus clementina* Peel essential oil | | *Citrus grandis* (grapefruit) peel essential oil | | *Citrus iyo* peel essential oil | | *Citrus junos* peel essential oil | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cold-pressed[13] | | Cold-pressed[14] | | Hydrodistilled[18,20] | | Cold-pressed[14] | | Hydrodistillation[17] | | Cold-pressed[6] | | Lyophilization/vacuum distillation[9] | | Cold-pressed[7] or not specified[16] | |
| | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max |
| β-Sesquiphellandrene | | | | | | | | | | | | | | | 0.1 | |
| β-Sinensal | Trace | 0.1 | | | | | | | | | Trace | | | | | |
| γ-Terpinene | Trace | 21.5 | | | 0.14 | 0.31 | | | | | | 0.005-0.05 | | | 11.4 | 12.3 |
| δ-Carene | | | | | | | | | | | | | | | ND | Trace |
| δ-Elemene | | | | | | | | | | | Trace | | | | 0.1 | 0.2 |
| δ-3-Carene | | | | | | 0.17 | | | | | | | | | | |
| Δ3-Carene | | | | | Trace | 0.01 | | | | | | | | | | |

| | *Citrus limon* (lemon) peel essential oils | | | | *Citrus nobilis* (mandarin orange) essential oil | | *Citrus sinensis* (orange) peel oil | | | | | | *Citrus reticulata* (tangerine) peel oil | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cold-pressed[12,13] | | Hydrodistilled[18] | | Cold-pressed[14] | | Cold-pressed[11,12,15,16] | | Hydrodistilled[44] | | Steam-distilled[22] | | Hydrodistilled[21,44] | |
| | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max |
| Essential oil content (%) | 1.1212 | | 1.318 | | | | 0.98-1.2112 | | 0.24-1.0744 | | 2.4922 | | 0.30-0.5044 | |
| Constituent | | | | | | | | | | | | | | |
| Limonene | 38.1 | 95.8 | 37.631 | 69.71 | | | 61.08 | 96.57 | 66.8 | 80.9 | | 77.49 | 64.1 | 92.4 |
| (*E*)-β-Farnesene | Trace | 0.2 | | | | | | | | | | | | |
| (*E*)-β-Ocimene | Trace | 0.4 | | | | | | 0.1 | | | | | | |
| (*E*)-Caryophyllene | Trace | 0.7 | | | | | | | | | | | | |
| (*E*)-Nerolidol | Trace | Trace | | | | | | | | | | | | |
| (Z)-β-Ocimene | Trace | 0.3 | Trace | 0.5 | | | | | | 0.34 | | | | 0.28 |
| Z-Carveol | | | | | | | | | 0.68 | 4.53 | | | | 1.29 |
| 1-Octanol | | | | | | | | | | | | | 0.55 | 0.95 |
| 1,3,8-*p*-Menthatriene | | | | | | | | | 1.21 | 1.69 | | | | |
| 1,8-Cineole | | | Trace | 0.82 | | | | | | | | | | |
| 2Z.6E-farnesol | | | Trace | | | | | | | | | | | |
| 3-Carene | Trace | Trace | | | | | | | | | | | | |
| 3-Furanacetic acid | | | | | | | | | | 3.0 | | | | |
| 3,3′,4′,5,6,7-Heptamethoxyflavone | | | | | X | | | | | | | | | |
| 3′,4′,5,6,7,8-Hexamethoxyflavone | | | | | X | | | | | | | | | |
| 4-Carene | | | | | | | | | 0.61 | 0.85 | | | | |
| 4-Carvon methanol | | | | | | | | | | | | | | 0.95 |
| 4-Terpineol | | | | | | | | | | | | 0.43 | | |
| 4-Vinyl guaiacol | | | | | | | | | 1.21 | 1.27 | | | 0.87 | 2.32 |
| 4′,5,6,7-tetramethoxyflavone | | | | | X | | | | | | | | | |
| 4′,5,6,7,8-Pentamethoxyflavone | | | | | X | | | | | | | | | |
| 6-Methylhept-5-en-2-one | Trace | Trace | | | | | | | | | | | | |
| 30 Carvacrol | | | Trace | 0.69 | | | | | | | | | | |
| Allyl isovalerate | | | | | | | | | | | | | | 0.36 |
| Benzaldehyde | | 0.11 | | | | | | | | | | | | |
| Bicyclogermacrene | | | | | | | | Trace | | | | | | |
| Borneol | | 0.16 | 0.33 | 2.54 | | | | 7.63 | | | | | | |
| Bornyl acetate | Trace | Trace | Trace | 0.01 | | | | | | | | | | |
| Camphene | Trace | 0.13 | Trace | 0.24 | | | | 0.32 | | | | | | |
| Camphor | | | Trace | 0.58 | | | | | | | | | | |
| Capraldehyde | | | | | | | 0.35 | 5.62 | | | | | | |
| Caprinaldehyde | | 0.26 | | | | | | 2.10 | | | | | | |

*(continued)*

**Table 4.** (continued)

| | *Citrus limon* (lemon) peel essential oils | | | | *Citrus nobilis* (mandarin orange) essential oil | | *Citrus sinensis* (orange) peel oil | | | | | | *Citrus reticulata* (tangerine) peel oil | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cold-pressed[12,13] | | Hydrodistilled[18] | | Cold-pressed[14] | | Cold-pressed[11,12,15,16] | | Hydrodistilled[44] | | Steam-distilled[22] | | Hydrodistilled[21,44] | |
| | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max |
| Caryophyllene oxide | Trace | 0.3 | Trace | | | | | | | | | | | |
| Carvone | | | | | | | | | | | | 0.17 | 0.47 | 0.62 |
| d-Carvone | | | | | | | | | | 0.39 | | | | |
| Cephrol | | | | | | | | | | | | 0.15 | | |
| *cis*-Carveol | | | | | | | | | | | | 0.22 | | |
| *cis*-Dihydrocarvone | | | Trace | 0.29 | | | | | | | | | | |
| *cis*-Limonene-1,2-oxide | Trace | 0.5 | | | | | | | | | | | | |
| *cis*-Linalool oxide THF | | | 0 | 0.49 | | | | | | | | | | |
| *cis*-Sabinene hydrate | | | Trace | 0.05 | | | | | | | | | | |
| *cis*-β-Farnesene | | | | | | | | | | | | | | 0.1 |
| *cis*-β-Ocimene | | | | | | | | 0.26 | | | | | | |
| Citral | | 0.27 | | | | | 1.74 | 7.74 | | | | | | |
| Citronellyl acetate | | | | | | | 0.01 | 0.22 | | | | | | |
| Citronellal | Trace | 0.4 | | | | | 0.06 | 0.2 | | | | | 0.45 | 0.78 |
| Citronellol | Trace | 0.15 | Trace | 0.04 | | | | 4.18 | 0.71 | 1.6 | | | 0.8 | 1.2 |
| Citronellyl acetate | Trace | 0.3 | | | | | | | | | | 0.12 | | |
| Copaene | | | | | | | | | | | | | | 0.89 |
| Decanal | Trace | Trace | | | | | | 0.21 | 0.71 | 1.02 | | 0.11 | 2.33 | 7.71 |
| Decanol | | | | | | | | 0.35 | | | | | | |
| Dehydrolinalool | | | | | | | | | | | | 0.40 | | |
| Dodecanol | | | | | | | | 0.03 | | | | | 0.42 | 0.65 |
| Elemol | | | | | | | | | | | | | 0.35 | 0.89 |
| Eugenol | | | | | | | | | | 0.53 | | | | |
| Farnesol | | | | | | | | | | | | | | 1.14 |
| Geranial | 0.1 | 2.9 | | | | | Trace | 0.11 | | | | | | |
| Geraniol | Trace | 0.2 | | | | | | | | | | | | |
| Geranyl acetate | Trace | 3.2 | Trace | 0.56 | | | | | | | | | | |
| Germacarene | | | | | | | | | | | | | 0.30 | 1.07 |
| Germacrene D | Trace | 0.8 | 0.019 | 1.35 | | | | 0.08 | | | | 0.27 | | |
| n-Heptane | | | | | | | | | | | | | | 0.4 |
| Hexadecane | | | | | | | | 0.2 | | | | | | |
| Isophorone | | | | | | | | | 1.09 | 2.92 | | | | |
| Isopropyl cresol | | | | | | | | | | | | | | 1.36 |
| Lemonol | | | | | | | | | | | | 0.28 | | |
| Limonene oxide | | | | | | | | | | 0.76 | | | | |
| Linalool | 0.1 | 25.1 | 0 | 1.59 | | | 0.12 | 2.32 | 1.52 | 2.1 | | 0.22 | 0.7 | 2.56 |
| Linalool oxide | | | | | | | | | 0.48 | 1.12 | | | | 0.6 |
| Linalyl acetate | Trace | 31.2 | Trace | 2.61 | | | Trace | | | | | 0.28 | | 0.4 |
| Longipinene | | | | | | | | | | | | | | 0.4 |
| Menthadien-1-ol | | | | | | | | | | | | | | 0.42 |
| Myrcene | 0.7 | 10.16 | Trace | 1.54 | | | | 2.5 | | | | 6.27 | | |
| Neral | Trace | 1.5 | | | | | | 0.06 | | | | | | |
| Nerol | Trace | 0.1 | Trace | 0.24 | | | | | | | | | | |
| Neryl acetate | 0.1 | 3.9 | | | | | | 0.04 | | | | | | |
| Nikkol | | | | | | | | | 5.7 | | | | | |
| Nonanal | Trace | 0.3 | Trace | | | | | 0.08 | | | | | | |
| n-Nonane | | | | | | | | 0.2 | | | | | | |
| Nootkatone | | | | | | | | 0.01 | | | | | 0.30 | 3.95 |
| Octanal | Trace | 0.1 | 0.02 | 1.59 | | | 0.34 | 0.8 | | | | | 1.06 | 1.15 |

*(continued)*

**Table 4.** (continued)

| | *Citrus limon* (lemon) peel essential oils | | | | *Citrus nobilis* (mandarin orange) essential oil | | *Citrus sinensis* (orange) peel oil | | | | | | *Citrus reticulata* (tangerine) peel oil | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cold-pressed[12,13] | | Hydrodistilled[18] | | Cold-pressed[14] | | Cold-pressed[11,12,15,16] | | Hydrodistilled[44] | | Steam-distilled[22] | | Hydrodistilled[21,44] | |
| | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max |
| Octyl acetate | Trace | 0.1 | | | | | | | | | | | | |
| *p*-Cymene | 0.1 | 7.8 | 0.23 | 9.84 | | | | | | | | | | |
| *p*-Cymenene | Trace | 0.3 | | | | | | | | | | | | |
| Pentadecane | | | | | | | | 0.2 | | | | | | |
| Perillaldehyde | | | | | | | | 0.03 | | | | | 1.64 | 1.65 |
| Piperitenone | | | | | | | | | | 0.42 | | | | |
| Sabinene | Trace | 6.3 | 3.8 | 6.48 | | | 0.21 | 0.5 | 0.37 | 0.62 | | 1.29 | | 0.49 |
| Spathulenol | Trace | 0.1 | Trace | | | | | | | | | | | |
| Terpineol-4 | Trace | 0.1 | | | | | | | | | | | | |
| Terpinen-4-ol | | | Trace | 1.28 | | | | | | | | | | 0.1 |
| Terpinolene | Trace | 0.8 | 0.02 | 0.22 | | | | 0.08 | | | | | | |
| Terpinyl acetate | | 0.5 | Trace | 0.15 | | | | | | | | | | |
| Tetradecane | | | | | | | | 0.2 | | | | | | |
| Thymol | Trace | 1.1 | | | | | | Trace | | | | | | |
| *trans*-α-Bergamotene | 0.2 | 1.3 | | | | | | | | | | | | |
| *trans*-Carveol | | | | | | | | | | | | 0.44 | | |
| *trans*-Limonene oxide | | | | | | | | 0.01 | | | | 0.20 | | |
| *trans*-Limonene-1,2-oxide | Trace | 0.6 | | | | | | | | | | | | |
| *trans*-Linalool oxide THF | Trace | Trace | | | | | | | | | | | | |
| *trans*-p-1,8-Dienol | | | | | | | | | | | | | | 0.52 |
| *trans*-*p*-menth-2-en-1-ol | Trace | 0.1 | | | | | | | | | | | | |
| *trans*-p-2,8-Menthadien-1-ol | | | | | | | | | 0.79 | 1.07 | | | | |
| *trans*-Sabinene hydrate | Trace | 0.1 | | | | | | | | | | | | |
| Tri-Cyclen | | | 0.02 | 0.16 | | | | | | | | | | |
| Valencene | | | Trace | 0.37 | | | | 0.05 | 0.41 | 1.2 | | | | |
| α-Bisabolene | | 0.2 | | | | | | | | | | | | |
| α-Cadinol | | | | | | | | | | | | | | 0.46 |
| α-Copaene | | | | | | | | 0.04 | | | | | | |
| α-Cubebene | | | | | | | | | | 0.7 | | | | 0.48 |
| α-Farnesene | | | | | | | | | | | | | 0.36 | 0.48 |
| α-Humulene | Trace | 0.2 | Trace | 0.04 | | | | | | | | | | |
| α-Phellandrene | Trace | 0.6 | | | | | | | | 0.46 | | | | |
| α-Pinene | 0.1 | 2.63 | 1.14 | 5.9 | | | 0.35 | 1.4 | 1.65 | 2.48 | | 1.49 | 0.6 | 1.67 |
| α-Sinensal | | | | | | | | | 0.35 | 0.49 | | | 0.93 | 5.0 |
| α-Terpinene | Trace | 0.4 | Trace | 1.05 | | | | | | | | | | |
| α-Terpineol | | | 0.93 | 1.7 | | | | | | | | | | 1.51 |
| α-Terpineol | Trace | 0.4 | | | | | | 0.07 | 2.34 | 3.1 | | 0.14 | 0.2 | 1.1 |
| α-Terpinolene | | 0.25 | | | | | 1.56 | 2.06 | | | | 0.38 | | 0.1 |
| α-Thujene | Trace | 1.7 | Trace | 0.38 | | | 0.04 | 0.11 | | | | 0.11 | | |
| α-Thujone | | | Trace | 0.35 | | | | | | | | | | |
| β-Bisabolene | 0.2 | 2.0 | | | | | | | | | | | | |
| β-Caryophyllene | | | | | | | | | | | | 0.21 | | 1.39 |
| β-Cubebene | | | | | | | | 0.03 | | | | | | 0.37 |
| β-Eleme | Trace | 0.5 | | | | | | 0.1 | | | | | | |
| β-Elemene | | | | | | | | | | | | 0.17 | | |
| β-Farnesene | | | | | | | | | | | | 3.64 | | |
| β-gurjurene | | | | | | | | 0.0 | | | | | | |
| β-Myrcene | | | | | | | | 1.88 | 3.76 | 4.41 | | | 3.27 | 4.05 |
| β-Ocimene | | | | | | | | | | | | 0.27 | | |
| β-Phellandrene | 0.1 | 4.2 | | | | | | | | | | | | 1.8 |

*(continued)*

**Table 4.** (continued)

| | *Citrus limon* (lemon) peel essential oils | | | | *Citrus nobilis* (mandarin orange) essential oil | | *Citrus sinensis* (orange) peel oil | | | | | | *Citrus reticulata* (tangerine) peel oil | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cold-pressed[12,13] | | Hydrodistilled[18] | | Cold-pressed[14] | | Cold-pressed[11,12,15,16] | | Hydrodistilled[44] | | Steam-distilled[22] | | Hydrodistilled[21,44] | |
| | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max |
| β-Pinene | 0.1 | 15.8 | 0.63 | 31.49 | | | Trace | 5.45 | | | | 0.41 | 0.2 | 0.4 |
| β-Sinensal | | | | | | | | | 0.33 | 0.83 | | | | 0.67 |
| γ-Eudesmol | | | | | | | | | | | | | | 1.08 |
| γ-Munrolene | | | | | | | | | | | | | | 1.16 |
| γ-Terpinene | Trace | 18.57 | 0.04 | 9.96 | | | 0.2 | 1.21 | | | | 3.34 | 0.23 | 2.6 |
| δ-Amorphene | | | | | | | | 0.05 | | | | | | |
| δ-Cadinene | | | | | | | | | | 0.32 | | | | 0.88 |
| δ-3-Carene | | | | | | | | 0.14 | | | | 0.69 | | |
| Δ3-Carene | 0.1 | 4.2 | Trace | | | | | | 0.78 | 1.03 | | | | |

Abbreviation: ND, none detected.

[a]Information on various cultivars and/or harvest times. X denotes these compounds were present; however, the amount present was not specified.

**Table 5.** Typical Levels of 5-Methoxypsoralen (5-MOP).

| Ingredient | 5-MOP level[26] |
|---|---|
| Tangerine oil cold-pressed | 50 ppm |
| Mandarin oil cold-pressed | 250 ppm |

**Table 6.** Levels of Major Coumarins and Furocoumarins in Lemon Oil and Lime Oil.

| Compound | % in lemon oil[8] | % in lime oil[8] | Photosensitizing activity[8] |
|---|---|---|---|
| 5-Geranoxypsoralen | 0.0387 | 2.2-2.5 | 0 |
| 5-Geranoxy-7-methoxycoumrin | 0.0603 | 2.2-5.2 | 0 |
| 5-Geranoxy-8-methoxypsoralen | Not analyzed | 0.945 | 0 |
| 5,7-Dimethoxycoumarin | 0.0295 | 0.464 | 0 |
| 5,8-Dimethoxypsoralen | Not analyzed | 0.508 | 0 |
| Oxypeucedanin | 0.005-0.073 | 0.0025 | + |
| 5-Methoxypsoralen | 0.0001-0.0087 | 0.17-0.33 | ++++ |

**Table 7.** Constituents That Are Established Contact Allergens in Humans, According to the SCCS.

| Constituent | Categorized according to number of patients reacting positively and to the number of patients tested (>1000 patients tested, unless indicated as rt, ie, rarely tested)[45] |
|---|---|
| β-Caryophyllene | ≤10 (oxidized and nonoxidized) |
| Carvone | ≤10 (rt) |
| Citral | 101-1000 |
| Citronellol | 11-100 |
| Coumarin | 101-1000 |
| Farnesol | 101-1000 |
| Geraniol | 101-1000 |
| Linalyl acetate | ≤10 |
| α- and β-pinene | 11-100 |
| (DL)-Limonene | 11-100 (nonoxidized); 101-1000 (oxidized) |
| Terpineol (mixture of isomers)/ α-terpineol | ≤10 |
| Terpinolene | 11-100 |

Abbreviations: rt, room temperature; SCCS, Scientific Committee on Consumer Safety.

cases, no reported uses were identified by the VCRP, but a maximum use concentration was provided in the industry survey. For example, *C grandis* (grapefruit) peel oil was not reported in the VCRP database to be in use, but the industry survey indicated that it is used at concentrations up to 0.05%. It should be presumed that *C grandis* (grapefruit) peel oil is used in at least 1 cosmetic formulation.

Table 9 lists the 7 citrus-derived peel oils not indicated to be in use based on the VCRP data and the results of the Council concentration of use survey.

Under the rules governing cosmetic products in the European Union, citrus-derived ingredients must have furocoumarin content below 1 mg/kg in sun protection products and in bronzing products.[25] The International Fragrance Association (IFRA) has issued standards for citrus oils and other furocoumarin-containing essential oils.[26] Thus, finished products that are applied to the skin, excluding rinse-off products like bath preparations and soaps, must not contain more than 0.0015%, or 15 ppm, 5-MOP. This equates to 0.0075%, or 75 ppm, in a fragrance compound used at 20% in a consumer product that is applied to the skin. If the level of 5-MOP has not been determined, limits specified for individual oils should be observed, and when such oils are used in combination with other phototoxic ingredients, the potential for an additive effect should be considered and use levels should be reduced accordingly. Restrictions for furocoumarin-containing essential oils have been recommended for bitter orange oil expressed, grapefruit oil expressed, lemon oil cold-pressed, and lime oil expressed.

An IFRA standard also has been issued for 7-methoxycoumarin, which is prohibited for use in fragrance compounds.[27] Based on established maximum levels of this substance from commercially available natural sources (like essential oils, extracts, and absolutes), exposure to 7-methoxycoumarin from the use of these oils and extracts is regarded to be acceptable if the level of 7-methoxycoumarin in the finished product does not exceed 100 ppm. An example of a maximum concentration based on this standard is 0.1% for lime cold-pressed oil.

Additionally, IFRA has set a standard stating that D-, L-, and DL-limonene and natural products containing substantial amounts of it should only be used when the level of peroxides is kept to the lowest practical level; such products should have a peroxide value of <20 mmol/L.[28] This standard is also cited by the European Commission.[29] The European Union also states that limonene must be included in the list of ingredients when its concentration exceeds 0.001% in leave-on products and 0.01% in rinse-off products.

The IFRA has also set limits on the amounts of some citrus-derived oils in finished products. For leave-on products applied to skin areas exposed to direct sunlight, these limits include 1.25% bitter orange peel expressed,[30] 4% grapefruit oil expressed,[31] 2% lemon oil cold-pressed,[32] and 0.7% lime oil expressed.[33] There are no restrictions for any of these oils in rinse-off products and products that are not applied to the skin. International Fragrance Association specified that if combinations of phototoxic fragrance ingredients are used, the use levels must be reduced accordingly, so that the sum of the concentrations of all phototoxic fragrance ingredients, expressed as a percentage of their respective recommended maximum levels, shall not exceed 100% in the consumer product. Additionally, the IFRA general standard described above for "Citrus oils and other furocoumarins-containing essential oils" must be considered.

**Table 8.** Frequency (2014) and Concentration of Use (2013) According to Duration and Type of Exposure for Citrus-Derived Ingredients.[23,24]

| | Number of use[46] | Maximum concentration of use (%) | Number of uses | Maximum concentration of use (%) | Number of uses | Maximum concentration of use (%) | Number of uses | Maximum concentration of use (%) |
|---|---|---|---|---|---|---|---|---|
| | *Citrus aurantium amara* (bitter orange) peel oil | | *Citrus aurantium dulcis* (orange) peel oil (Listed as *Citrus sinensis* (sweet orange) peel oil in VCRP) | | *Citrus grandis* (grapefruit) peel oil | | *Citrus junos* peel oil | |
| Totals[a] | 127 | 0.05-2 | 289 | 0.00002-29 | NR | 0.00004-0.05 | 6 | NR |
| Duration of use | | | | | | | | |
| Leave-on | 74 | 0.2-2 | 156 | 0.00038-0.54 | NR | 0.0004-0.0008 | 4 | NR |
| Rinse-off | 45 | 0.05-0.25 | 110 | 0.00002-29 | NR | 0.00004-0.05 | 2 | NR |
| Diluted for (bath) use | 8 | NR | 23 | 0.33 | NR | 0.0014 | NR | NR |
| Exposure type | | | | | | | | |
| Eye area | 2 | NR | 3 | 0.1 | NR | NR | NR | NR |
| Incidental ingestion | 1 | 0.75 | 4 | NR | NR | NR | NR | NR |
| Incidental inhalation—Spray | 27[b]; 28[c] | NR | 56[b]; 55[c] | 0.00038[b] | NR | NR | 2 | NR |
| Reported spray | NR | NR | NR | NR | NR | NR | NR | NR |
| Incidental inhalation—Powder | 28[c] | 0.9-2[d] | 3[d]; 55[c] | 0.03-0.4[d] | NR | 0.0004[d] | 1 | NR |
| Reported powder | NR | NR | NR | NR | NR | NR | NR | NR |
| Dermal contact | 115 | 0.05-2 | 218 | 0.001-0.4 | NR | 0.00004-0.05 | 6 | NR |
| Deodorant (underarm) | NR | NR | 1[b] | NR | NR | NR | NR | NR |
| Reported spray | NR | NR | NR | NR | NR | NR | NR | NR |
| Reported as not spray | NR | NR | NR | NR | NR | NR | NR | NR |
| Hair—Noncoloring | 11 | NR | 65 | 0.00002-29 | NR | 0.005 | NR | NR |
| Hair—Coloring | NR | NR | NR | NR | NR | NR | NR | NR |
| Nail | NR | NR | 2 | 0.5-0.54 | NR | NR | NR | NR |
| Mucous membrane | 21 | 0.25-0.75 | 56 | 0.1-0.33 | NR | 0.0014 | 1 | NR |
| Baby products | 6 | NR | 8 | NR | NR | NR | NR | NR |

| | Number of use[46] | Maximum concentration of use (%) | Number of uses | Maximum concentration of use (%) | Number of uses | Maximum concentration of use (%) | Number of uses | Maximum concentration of use (%) |
|---|---|---|---|---|---|---|---|---|
| | *Citrus limon* (lemon) peel oil | | *Citrus nobilis* (mandarin orange) peel oil | | *Citrus reticulata* (mandarin orange) peel oil[e] | | *Citrus sinensis sanguinello* (blood orange) peel oil[e] | |
| Totals[a] | 490 | 0.0001-0.5 | 152 | 0.00005-0.1 | 2 | NR | 8 | NR |
| Duration of use | | | | | | | | |
| Leave-on | 297 | 0.0001-0.5 | 86 | 0.00005-0.1 | 1 | NR | 4 | NR |
| Rinse-off | 165 | 0.0006-0.001 | 58 | 0.00005-0.03 | 1 | NR | 4 | NR |
| Diluted for (bath) use | 28 | 0.012 | 8 | NR | NR | NR | NR | NR |
| Exposure type | | | | | | | | |
| Eye area | 9 | NR | NR | NR | NR | NR | NR | NR |
| Incidental ingestion | 8 | NR | 1 | 0.0099 | NR | NR | NR | NR |
| Incidental inhalation—Spray | 84[b]; 96[c] | 0.06[c] | 45[b]; 26[c] | NR | 1[c] | NR | 1[b]; 2 | NR |
| Reported spray | NR | NR | NR | NR | NR | NR | 1 | NR |
| Incidental inhalation—Powder | 1[d]; 96[c] | 0.001-0.14[d]; 0.06[c] | 26[c] | 0.00005-0.14 | 1[c] | NR | 2[c] | NR |
| Reported powder | NR | NR | NR | NR | NR | NR | NR | NR |

*(continued)*

**Table 8.** (continued)

| | Number of use[46] | Maximum concentration of use (%) | Number of uses | Maximum concentration of use (%) | Number of uses | Maximum concentration of use (%) | Number of uses | Maximum concentration of use (%) |
|---|---|---|---|---|---|---|---|---|
| | *Citrus limon* (lemon) peel oil | | *Citrus nobilis* (mandarin orange) peel oil | | *Citrus reticulata* (mandarin orange) peel oil[e] | | *Citrus sinensis sanguinello* (blood orange) peel oil[e] | |
| Dermal contact | 411 | 0.0001-0.5 | 136 | 0.00005-0.1 | 2 | NR | 8 | NR |
| Deodorant (underarm) | NR | NR | NR | NR | NR | NR | NR | NR |
| Reported spray | NR | NR | NR | NR | NR | NR | NR | NR |
| Reported as not spray | NR | 0.002 | NR | NR | NR | NR | NR | NR |
| Hair—Noncoloring | 67 | NR | 15 | 0.00005-0.03 | NR | NR | NR | NR |
| Hair—Coloring | NR | NR | NR | NR | NR | NR | NR | NR |
| Nail | 3 | 0.0001-0.14 | NR | 0.012 | NR | NR | NR | NR |
| Mucous membrane | 96 | 0.001-0.012 | 40 | 0.0099 | NR | NR | 4 | NR |
| Baby products | 8 | NR | NR | NR | NR | NR | NR | NR |

| | Number of use[46] | Maximum concentration of use (%) | Number of uses | Maximum concentration of use (%) | Number of uses | Maximum concentration of use (%) | Number of uses | Maximum concentration of use (%) |
|---|---|---|---|---|---|---|---|---|
| | *Citrus tangerina* (tangerine) peel oil | | | | | | | |
| Totals[a] | 34 | 0.0000013 | | | | | | |
| Duration of use | | | | | | | | |
| Leave-on | 18 | 0.0000013 | | | | | | |
| Rinse-off | 15 | 0.0000013 | | | | | | |
| Diluted for (bath) use | 1 | NR | | | | | | |
| Exposure type | | | | | | | | |
| Eye area | NR | NR | | | | | | |
| Incidental ingestion | 1 | NR | | | | | | |
| Incidental inhalation—Spray | 5[b]; 8[c] | 0.0000013b | | | | | | |
| Reported spray | 1 | NR | | | | | | |
| Incidental inhalation—Powder | 8[c] | NR | | | | | | |
| Reported powder | NR | NR | | | | | | |
| Dermal contact | 26 | NR | | | | | | |
| Deodorant (underarm) | NR | NR | | | | | | |
| Reported spray | NR | NR | | | | | | |
| Reported as not spray | NR | NR | | | | | | |
| Hair—Noncoloring | 7 | 0.0000013 | | | | | | |
| Hair—Coloring | NR | NR | | | | | | |
| Nail | NR | NR | | | | | | |
| Mucous membrane | 7 | NR | | | | | | |
| Baby products | NR | NR | | | | | | |

Abbreviations: INCI, International Nomenclature of Cosmetic Ingredient; NR, not reported; VCRP, Voluntary Cosmetic Registration Program.
[a]Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.
[b]It is possible these products may be sprays, but it is not specified whether the reported uses are sprays.
[c]Not specified whether a powder or a spray, so this information is captured for both categories of incidental inhalation.
[d]It is possible these products may be powders, but it is not specified whether the reported uses are powders.
[e]Use in a spray product has been reported in response to a survey conducted by the Council.

**Table 9.** Ingredients That Are Not Reported to Be in Use.

| |
|---|
| *Citrus aurantifolia* (lime) peel oil |
| *Citrus aurantium currassuviensis* peel oil |
| *Citrus clementina* peel oil |
| *Citrus iyo* peel oil |
| *Citrus medica vulgaris* peel oil |
| *Citrus reticulata* (Tangerine) peel oil |
| *Citrus tachibana/reticulata* peel oil |

### *Noncosmetic*

The essential oils, oleoresins (solvent-free), and natural extractives (including distillates) derived from the following citrus fruits are GRAS for their intended use in foods for human consumption: *C aurantifolia* (lime), *C aurantium* (bitter orange; the flowers and peel), *C limon* (lemon), *C reticulata* (tangerine), *Citrus reticulata blanco* (mandarin), *C sinensis* (orange; the leaf, flowers, and peel), and citrus peels (species not specified; 21CFR182.20). These essential oils, oleoresins (solvent-free), and natural extractives (including distillates) of these citrus fruits are GRAS for their intended use in animal drugs, feeds, and related products (21CFR582.20).

Citrus essential oils are used in the pharmaceutical industry as flavoring agents to mask the unpleasant taste of drugs.[18] *Citrus aurantium amara* (bitter orange) and extracts of its dried fruit and peel have been used in traditional Western medicines and in Chinese and Japanese herbal medicines.[34]

## Toxicological Studies

As noted earlier, the citrus ingredients in this assessment are found in foods, and daily exposures from food use would result in a much larger systemic exposure than those from use in cosmetic products. Essential oils, oleoresins (solvent-free), and natural extracts (including distillates) derived from some citrus fruits are GRAS for their intended use in foods for human and animal consumption according to the FDA. Volatile oils of limes, lemons, grapefruits, bitter oranges, oranges, and tangerines are described as flavoring agents in the USP Food Chemicals Codex.[1] Therefore, the systemic toxicity potential of these ingredients is not addressed further in this report. The primary focus of this safety assessment is on the potential for irritation and sensitization from dermal exposure to these citrus ingredients as used in cosmetic products.

### *Acute Toxicity*

#### *Dermal—Nonhuman*

*Lemon oil.* The dermal $LD_{50}$ of lemon oil was greater than 10 g/kg in rabbits.[3] An occlusive patch of undiluted oil was applied to the skin of 6 animals for 24 hours. One animal died during the observation period.

*Mandarin oil—Expressed (C reticulata).* The dermal $LD_{50}$ of mandarin peel oil (C reticulata) was greater than 5 g/kg in rabbits.[10] An occlusive patch of undiluted oil was applied to the skin of seven animals for 24 hours.

*Orange oil.* The dermal $LD_{50}$ of sweet orange oil was greater than 5 g/kg in female New Zealand White rabbits.[4] An occlusive patch of undiluted oil was applied to the skin of 10 animals for 24 hours. Signs of skin irritation were reported, including moderate redness in 10 of 10 animals, slight edema in 3 of 10 animals, and moderate edema in 5 of10 animals.

## Reproductive and Developmental Toxicity

No published reproductive and developmental studies on citrus-derived peel oils were identified in a literature search for these ingredients, and no unpublished data were submitted.

## Genotoxicity

Genotoxicity studies for in vitro assays are summarized in Table 10. No genotoxic effects were observed for lemon oil or sweet orange oil in bacterial reverse mutation assays, mouse lymphoma cell mutation assays, and Chinese hamster chromosome aberration assays.[3,4]

## Carcinogenicity

### *Orange Oil, Lemon Oil, Grapefruit Oil, and Lime Oil*

Tumor-promoting activity was observed in mouse skin exposed to essential oils of orange (sweet), lemon, grapefruit, or lime.[35] Chemical constituents of these oils were not fully identified in this study, although the terpene and nonterpene fractions were separated. Groups of 10 male and 10 female strain 101 mice received a single application of 9,10-dimethyl-1,2-benzanthracene (DMBA) in acetone (300 μg in 0.2 mL in 4 groups, 225 μg in 0.15 mL in a fifth group). Group 1 was a control group that received no further treatments. Groups 2 through 5 received weekly applications of 0.25 mL of the test substances 3 weeks after the application of DMBA.

By the fifth week, papillomas were observed in group 3 (lemon oil), group 4 (grapefruit oil), and group 5 (lime oil). Papillomas were observed in group 2 (orange oil) by the 12th week. After 33 weeks, 10 of 20 mice in the lemon oil and lime oil treatment groups and 13 of 20 mice in the grapefruit oil and orange oil groups had papillomas. Only 1 mouse in the control group had papillomas after 33 weeks, and the affected site was not the treated skin. Additionally, one female mouse of the lemon oil group developed a sebaceous-gland tumor of the nipple. No malignant skin tumors were observed in the orange oil group: Treatment for this group was stopped after 42 weeks. Squamous cell carcinomas of the skin were observed in 2 mice from the lemon oil group and2 mice of the grapefruit oil group between weeks 36 and 55. One malignant skin tumor was observed in the lime oil group at week 34; however, the mouse was found dead and a proper histological examination was not possible. No malignant skin tumors were observed in the control group. Nondermal tumors during the treatment period were observed in one mouse of the orange oil group (a hemangioma

**Table 10.** Genotoxicity.

| Test article | Concentration/ dose | Procedure | Results | Reference |
|---|---|---|---|---|
| In vitro | | | | |
| Lemon oil in ethanol | 10-5000 μg/plate | Reverse mutation assay using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2uvrA, with and without S9 metabolic activation | Toxicity was observed in all test strains except WP2uvrA with and without S9; no significant dose-related increases in the number of revertant colonies in any test strain at any dose level, with or without metabolic activation; controls yielded expected results; lemon oil was not mutagenic in this assay | [3] |
| Lemon oil in ethanol | 40-100 μg/mL | Cell mutation assay in mouse lymphoma L5178Y TK ± cells in accordance with OECD guideline 476 in 2 independent experiments; with and without S9 metabolic activation | No significant increases in the mutation frequency were observed in either experiment, with or without S9 activation; controls yielded the expected results; lemon oil did not induce gene mutations in mouse lymphoma cells | [3] |
| Lemon oil in ethanol | Up to 0.125 mg/mL | Chromosome aberration study using Chinese hamster lung fibroblasts (CHL) in accordance with OECD guideline 473, without metabolic activation, 100 metaphases examined | The incidence of polyploidy cells at 24 hours post-treatment was 1.0%, and the incidence of cells with structural chromosome aberrations at 24 hours after treatment was 2.0%; the test material did not significantly induce chromosomal aberrations in CHL cells; lemon oil was not considered clastogenic | [3] |
| Sweet orange oil in ethanol | 1-5000 μg/plate | Reverse mutation assay using *S typhimurium* strains TA98, TA100, TA1535, and TA1537 and *E coli* strain WP2uvrA, with and without S9 metabolic activation | Cytotoxicity was observed in all test strains except WP2uvrA with and without S9; no significant dose-related increases in the number of revertant colonies in any test strain at any dose level, with or without metabolic activation; controls yielded expected results; sweet orange oil was not mutagenic in this assay | [4] |
| Sweet orange oil in ethanol | 40-100 μg/mL | Cell mutation assay in mouse lymphoma L5178Y TK ± cells in accordance with OECD guideline 476 in 2 independent experiments; with and without S9 metabolic activation | The test material did not induce a significant increase in the mutation frequency in both experiments, with or without metabolic activation; sweet orange oil did not induce gene mutations in mouse lymphoma cells | [4] |
| Sweet orange oil in ethanol | Up to 0.125 mg/mL | Chromosome aberration study using CHL fibroblasts in accordance with OECD guideline 473, without metabolic activation, 100 metaphases examined | The incidence of polyploidy cells at 48 hours post-treatment was 1.0%, and the incidence of cells with structural chromosome aberrations at 48 hours after treatment was 1.0%; the test material did not significantly induce chromosomal aberrations in CHL cells; sweet orange oil was not considered clastogenic | [4] |

Abbreviation: OECD, Organisation for Economic Co-operation and Development.

of the subcutaneous tissue starting at week 7) and in one mouse of the grapefruit oil group (a spindle cell sarcoma of the subcutaneous tissues). No tumors of the internal organs were observed. The survival of all the mice, including the controls, in this experiment was poor because of a very high incidence of renal disease.[35]

## *Orange Oil*

Tumor-promoting activity was observed in mouse skin exposed to orange (sweet) oil.[35] In the study, groups of 10 male and 10 female strain 101 mice received a single application of DMBA in acetone (300 μg in 0.15 mL). One group (15 mice of each

**Table 11.** Dermal Irritation Studies for Citrus-Derived Ingredients.

| Test article | Concentration/ dose | Test population | Procedure | Results | Reference |
|---|---|---|---|---|---|
| Nonhuman | | | | | |
| Lemon oil | 5 mL/kg | 6 New Zealand White rabbits | Acute dermal toxicity limit test scored under the Draize method; 24-hour occlusive patches on intact and abraded skin | Irritating | 3 |
| Orange oil, cold-pressed | 0.5 mL of undiluted material | 3 male albino rabbits | Skin irritation study conducted according to OECD guideline 404; semi-occluded patch for 4 hours | Mean erythema/eschar scores were 2.0, 1.7, and 2.0; mean edema scores were 2.0, 1.3, and 1.3; irritating to skin | 4 |
| Mandarin peel oil, expressed (described as *Citrus reticulata*) | 5 mg/kg | 7 rabbits | 24-hour occlusive, single dose study | Slight erythema and edema | 10 |
| Mandarin peel oil, expressed (described as *C reticulata*) | Not reported | Hairless mice and miniature swine; details not provided | Open patch tests; details not provided | 2 of 3 samples were irritating | 10 |
| Human | | | | | |
| Lemon oil | 0.3%, 2%, or 20%; multiple vehicles | 34 patients at 0.3%, 30 subjects at 2%, and 35 patients at 20% | 24-72 hours occlusive patch tests | No irritation at 0.3% and 20%, 1 $\pm$ reaction at 2% | 36 |
| Mandarin peel oil, expressed (described as *C reticulata*) | 8% in petrolatum | 5 patients | 48 hours closed patch test; details not provided | No irritation | 10 |

Abbreviation: OECD, Organisation for Economic Co-operation and Development.

sex) was a control group that received no further treatments. Two groups received weekly applications of 0.25 mL of 40% orange oil in acetone or 80% orange oil in acetone 3 weeks after the initial application of DMBA. The applications continued for 37 weeks.

Papillomas were observed in both groups treated with orange oil starting on the 12th week. After 33 weeks, 5 of 10 mice treated with 40% orange oil and 10 of 10 mice treated with 80% orange oil had papillomas, and at study end, only 1 tumor of each group was found outside the treated area. Four mice in the control group had papillomas by week 33, but these tumors were outside the treated area of the skin. Malignant tumors were observed in one mouse of each treatment group, arising from the preexisting papilloma. Both tumors were squamous cell carcinomas, infiltrating the panniculus muscle. Additionally, tumors of the urethral orifice were observed in 4 female mice of the 40% orange oil group. The survival of all the mice in this experiment was poor due to a very high incidence of renal disease.

In the same study, tumor-promoting activity was observed in mice exposed to undiluted orange oil after pretreating the mice with either dermal or intraperitoneal injections of urethane. The effect was weak compared to the effects observed after DMBA induction.

A similar experiment performed in the same study tested the carcinogenic effects of orange oil without pretreatment with DMBA or urethane. The mice were treated once weekly with 0.25 mL of 40% or 80% orange oil in acetone or undiluted orange oil for 38 (diluted) or 46 (undiluted) weeks. This study found no evidence of direct tumorigenic effects on the treated mouse skin. Urethral orifice tumors were observed in one female mouse of the 40% orange oil group and in one female mouse of the 80% orange oil group. A papilloma was observed on the head of a mouse (outside the treatment site) that was treated with 80% orange oil.[35]

## Irritation and Sensitization

### Dermal Irritation

Dermal irritation studies are summarized in Table 11. Lemon oil, orange oil, and mandarin peel oil all produced some reaction in irritation studies in animals, but in human patients, no irritation was observed after topical exposure to lemon oil (up to 20%) or mandarin peel oil (8%).[3,4,10,36]

### Ocular Irritation

*Lemon oil.* Lemon oil tested at 5% was not irritating to the eyes of 3 albino rabbits.[3] Each rabbit had 0.1 mL of the test material

**Table 12.** Sensitization Studies for Citrus-Derived Ingredients.

| Test article | Concentration/ dose | Test population | Procedure | Results | Reference |
|---|---|---|---|---|---|
| Human: Patch | | | | | |
| Bitter orange oil | 2% in paraffin | 200 patients with dermatitis tested with 35 essential oils plus an additional 50 patients with balsam sensitivity | Sensitization patch study, details not provided | 6 positive reactions | 37 |
| Lemon oil | 2% in paraffin | 200 patients with dermatitis tested with 35 essential oils plus an additional 50 patients with balsam sensitivity | Sensitization patch study, details not provided | 4 positive reactions, details not provided | 37 |
| Lemon oil | Not reported | 100 patients | Marzulli-Maibach sensitization technique; open patches | 2% of the patients had a positive skin reaction at the first reading after challenge, no reactions were noted at the 48 and 72 hours readings, study concluded the test material was not sensitizing | 3 |
| Mandarin oil, expressed (described as *Citrus reticulate*) | 8% in petrolatum | 25 patients | Maximization study, details not provided | Not sensitizing | 10 |
| Sweet orange oil | 2% in paraffin | 200 patients with dermatitis tested with 35 essential oils plus an additional 50 patients with balsam sensitivity | Sensitization patch study, details not provided | 3 positive reactions, details not provided | 37 |
| Sweet orange oil | Not reported | 100 patients | Marzulli-Maibach sensitization technique; open patches | 4% of the patients had positive skin reaction at the first reading after challenge, no reactions were noted at the 48 and 72 hours readings, study concluded the test material was not sensitizing | 4 |

instilled into the right eye with no further treatment. The left eye served as the control. Eyes were examined every 24 hours for 4 days and then again on the seventh day. No corneal opacity or iris congestion was observed. An intense conjunctival irritation involving chemosis and discharge occurred. Treated eyes were normal on the seventh day.

*Orange oil.* Orange oil tested undiluted did not induce significant or irreversible damage to the eyes of 3 male New Zealand White rabbits.[4] The test was performed in accordance with Organisation for Economic Co-operation and Development Test Guideline 405. The test material (0.1 mL) was instilled into one eye of each rabbit. The mean scores calculated for the 3 animals across 3 scoring times were 0.0 for corneal opacity and iris lesions and 1.0 for reddening of the conjunctivae.

## Sensitization

Sensitization studies are presented in Table 12. Mandarin peel oil (8% in petrolatum) was not sensitizing in human maximization tests. In studies of 250 patients with dermatitis, less than 2.5% had positive reactions to bitter orange oil, lemon oil, or sweet orange oil tested at 2% in paraffin.[3,4,10,37]

## Phototoxicity and Photosensitization

Phototoxicity and photosensitization studies are presented in Table 13. Phototoxic potential was observed for sweet orange oil and lemon oil in in vitro studies. No phototoxic responses were noted in some animal studies of lime oil, lemon oil, grapefruit oil, mandarin oil, or tangerine oil; however, signs of phototoxicity were observed in response to undiluted lime oil and lime oil diluted to concentrations of 15%, undiluted bitter orange oil, and undiluted lemon oil and lemon oil diluted to concentrations of 50%. In human studies, phototoxic reactions were observed in response to undiluted bitter orange oil, lemon oil (1%), sweet orange oil (1%), and undiluted and diluted expressed lime oil (30%). Many of the citrus-derived peel oils contain constituents that are photoactive agents, although those noted to be furocoumarin free tended not to induce photosensitization.[19,30,38-41]

## Occupational Exposure

In a retrospective study (2001-2010) of professional food handlers in Denmark, 8.5% (16 of 188) of the patients had positive skin prick test reactions to orange peel and 7.9% (15 of 191) of

**Table 13.** Photosensitization and Phototoxicity Studies.

| Test article | Concentration/dose[a] | Test population | Procedure | Results | Reference |
|---|---|---|---|---|---|
| Alternative studies | | | | | |
| Sweet orange oil, including deterpenated kind | Concentrations not reported; tested in PBS, ethanol, or DMSO with samples from 3 suppliers | 3T3 Balb/c fibroblasts | - 3T3 neutral red uptake phototoxicity test<br>- Light source was a doped mercury-metal halide lamp, filtered with 50% transmission at 335 nm to diminish UVB | Borderline phototoxic, positive phototoxic results observed more frequently with the vehicles PBS and ethanol with certain supplied samples | 38 |
| Lemon oil, including deterpenated kind | Same as above | Same as above | Same as above | Borderline phototoxic, positive phototoxic results observed in all 3 vehicles, but were more prominent in the deterpenated sample | 38 |
| Sweet orange oil, including deterpenated kind | Up to 3.16% in water with samples from 3 suppliers | Same as above | Same as above | Potential for phototoxicity observed | 38 |
| Lemon oil, including deterpenated kind | Same as above | Same as above | Same as above | Cytotoxicity observed with deterpenated lemon oil; potential for phototoxicity observed | 38 |
| Nonhuman | | | | | |
| Lime oil, distilled (psoralen-free) | Undiluted; 20 μL | Hairless mice, 6/group | - A single dose was applied to a 2 $cm^2$ area on the back; animals were exposed to irradiation 30 minutes after dosing<br>- One group was exposed to a compact-arc xenon lamp for 2 minutes (wavelengths $<$295 nm or 320-280 nm excluded)<br>- One group was exposed to a long-arc xenon lamp for 40 minutes at a distance of 1 m; the weighted energy was 0.1667 $W/m^2$<br>- One group was exposed to 4 fluorescent black light lamps (UVB eliminated) for 1 hour at an integrated UVA intensity of 3 $W/m^2$<br>- Positive controls were treated with 0.01% 8-methoxypsoralen in methanol; negative controls with an appropriate vehicle<br>- Test sites were examined 4, 24, 48, 72, and 96 hours after exposure | - A phototoxic response was not observed with any of the light sources | 40 |
| Lime oil, distilled (psoralen-free) | Undiluted; 20 μL | Miniature swine, 2/group | As above | - A phototoxic response was not observed with any of the light sources | 40 |
| Lime oil, expressed | Undiluted and diluted; 20 μL | Hairless mice, 6/group | As above | - A phototoxic response was observed with all 3 light sources<br>- The lowest phototoxic concentration was 15% | 40 |
| Lime oil, expressed | Undiluted and diluted; 20 μL | Miniature swine, 2/group | As above | - A phototoxic response was observed with all 3 light sources<br>- The lowest phototoxic concentration was 30% | 40 |
| Grapefruit oil | Undiluted; 20 μL | 6 hairless mice and 2 miniature swine | - As above | Not photosensitizing | 40 |

*(continued)*

**Table 13.** (continued)

| Test article | Concentration/dose[a] | Test population | Procedure | Results | Reference |
|---|---|---|---|---|---|
| Lime oil distilled (psoralen-free) | Undiluted; 20 μL | 6 hairless mice and 2 miniature swine | - As above | Not photosensitizing | [40] |
| Lime oil; expressed and rectified | Undiluted; 20 μL | 6 hairless mice and 2 miniature swine | - As above | Not photosensitizing | [40] |
| Lime oil Persian Florida; expressed and rectified | Undiluted; 20 μL | 6 hairless mice and 2 miniature swine | - As above | Not photosensitizing | [40] |
| Mandarin oil | Undiluted; 20 μL | 6 hairless mice and 2 miniature swine | - As above | Not photosensitizing | [40] |
| Mandarin oil, Italian | Undiluted; 20 μL | 6 hairless mice and 2 miniature swine | - As above | Not photosensitizing | [40] |
| Oil of lemon, California | Undiluted; 20 μL | 6 hairless mice and 2 miniature swine | - As above | Not photosensitizing | [40] |
| Oil of lemon, distilled | Undiluted; 20 μL | 6 hairless mice and 2 miniature swine | - As above | Not photosensitizing | [40] |
| Oil mandarin, Italian | Undiluted; 20 μL | 6 hairless mice and 2 miniature swine | - As above | Not photosensitizing | [40] |
| Oil of tangerine | Undiluted; 20 μL | 6 hairless mice and 2 miniature swine | - As above | Not photosensitizing | [40] |
| Orange oil; cold-pressed | Undiluted; 20 μL | 6 hairless mice and 2 miniature swine | - As above | Not photosensitizing | [40] |
| California lemon oil | Undiluted; 20 μL | 6 hairless mice and 2 miniature swine | - As above | - A phototoxic response was observed | [40] |
| Italian lemon oil | Undiluted; 20 μL | 6 hairless mice and 2 miniature swine | - As above | - A phototoxic response was observed | [40] |
| Oil lemon, Greek; cold-pressed | Undiluted; 20 μL | 6 hairless mice and 2 miniature swine | - As above | - A phototoxic response was observed | [40] |
| Oil lemon, Italian | Undiluted; 20 μL | 6 hairless mice and 2 miniature swine | - As above | - A phototoxic response was observed | [40] |
| Oil lemon, IC (not defined) | Undiluted; 20 μL | 6 hairless mice and 2 miniature swine | - As above | - A phototoxic response was observed | [40] |
| Lime oil, expressed | Undiluted; 20 μL | 6 hairless mice and 2 miniature swine | - As above | - A phototoxic response was observed | [40] |
| Oil limes, Persian | Undiluted; 20 μL | 6 hairless mice and 2 miniature swine | - As above | - A phototoxic response was observed | [40] |
| Oil limes, expressed and rectified | Undiluted; 20 μL | 6 hairless mice and 2 miniature swine | - As above | - A phototoxic response was observed | [40] |
| Lime oil, expressed and rectified | Undiluted; 20 μL | 6 hairless mice and 2 miniature swine | - As above | - A phototoxic response was observed | [40] |
| Bitter orange oil | Undiluted; 20 μL | 6 hairless mice and 2 miniature swine | - As above | - A phototoxic response was observed | [40] |
| Lemon oil from multiple regional sources | 20%, 50%, or 100% in ethanol; 0.02 mL | Albino guinea pigs | - The oil was applied to the shaved back of the animals<br>- The animals were then exposure to UVA radiation (320-400 nm, 13 J/cm$^2$) | - Concentrations of 50% and 100% elicited phototoxicity in most of the samples tested<br>- Lemon oils from different regions had different phototoxicity potencies | [41] |

*(continued)*

**Table 13.** (continued)

| Test article | Concentration/dose[a] | Test population | Procedure | Results | Reference |
|---|---|---|---|---|---|
| | | | - Erythema was evaluated 24, 48, and 72 hours after irradiation<br>- The samples were then fractionated subsequent phototoxicity testing of the isolated components was performed | - Oxypeucedanin and 5-methoxypsoralen (furocoumarins) were identified as phototoxic | |
| Human | | | | | |
| Bitter orange peel oil | Undiluted; 5 $\mu L/cm^2$ | 8 patients | - An occlusive patch was applied to a 2 cm $\times$ 2 cm area<br>- 1 cm site on each patient was exposed to visible light of 20 $J/cm^2$ UVA<br>- The test sites were scored after 24 and 48 hours | All patients reacted (details not provided) | 30 |
| Lemon oil, including deterpenated kind | Up to 1% in water from samples from 3 supplier; dose not provided | 5 female patients | -2 occlusive 10 mm diameter Finn Chambers on both sides of the lower back<br>- Exposure time to test material was 1 hour<br>- Irradiation immediately to 1 site after patch removal at a dose of 5 $J/cm^2$as measured in the UVA range<br>- Test sites scored after 24, 48, and 72 hours<br>- Light source was a doped mercury-metal halide lamp, filtered with 50% transmission at 335 nm to diminish UVB | - Phototoxic reactions concurrent with an irritation reactions were observed in lemon oil at 1% in 4 of 5 patients up to 72 hours after irradiation<br>- Phototoxic reactions were observed in deterpenated lemon oil at 0.1% in 2 of 5 patients at 48 and 72 hours after irradiation<br>- No reactions were observed at concentrations of 0.1% or lower in lemon oil and 0.01% in deterpenated lemon oil | 38 |
| Sweet orange oil, including deterpenated kind | Same as above | Same as above | - Same as above | - Phototoxic reaction were observed in orange oil at 1% in 3 of 5 patients at 24 hours and 2 of 5 patients at 48 and 72 hours after irradiation<br>- No reactions were observed at concentrations of 0.1% or lower in orange oil or 0.1% and 0.01% in deterpenated orange oil | 38 |
| Lime oil, distilled | Undiluted | 10 Caucasian patients | - A single dose was applied to a 2-$cm^2$ area on the back<br>- 30 minutes after dosing, patients were exposed to sunlight for 30 minutes or a compact-arc xenon lamp for 2 minutes (wavelengths $<$295 nm or 320-280 nm excluded)<br>- Positive controls were treated with 0.01% 8-methoxypsoralen in methanol; negative controls with an appropriate vehicle<br>- Test sites were examined 4, 24, 48, 72, and 96 hours after exposure | No phototoxic response was observed | 40 |

*(continued)*

**Table 13.** (continued)

| Test article | Concentration/dose[a] | Test population | Procedure | Results | Reference |
|---|---|---|---|---|---|
| Lime oil, expressed | Undiluted and diluted; 20 μL | 10 Caucasian patients | - A single dose was applied to a 2 $cm^2$ area on the back<br>- 30 minutes after dosing, 1 treated site and the control untreated site were exposed to sunlight for 30 minutes, a compact-arc xenon lamp for 2 minutes (wavelengths <295 nm or 320-280 nm excluded), or 4 fluorescent black light lamps (UVB eliminated) for 1 hour at an integrated UVA intensity of 3 $W/m^2$<br>- Positive controls were treated with 0.01% 8-methoxypsoralen in methanol; negative controls with an appropriate vehicle<br>- Test sites were examined 4, 24, 48, 72, and 96 hours after exposure | - A phototoxic response was observed with all 3 light sources<br>- The lowest phototoxic concentration with the simulated light sources was 30% | [40] |

Abbreviations: DMSO, dimethyl sulfoxide; PBS, phosphate-buffered saline; UVA, ultraviolet A; UVB, ultraviolet B.
[a]The solvent is specified when known.

the patients had positive skin prick test reactions to lemon peel.[42]

## Summary

The 14 citrus-derived peel oils described in this report function primarily as skin conditioning agents-miscellaneous and fragrance. Botanicals such as citrus are composed of hundreds of constituents, some of which have the potential to cause toxic effects; for example, bergapten (as known as 5-MOP) is a naturally occurring, phototoxic furanocoumarin (psoralen) in citrus peel oils. Presently, CIR reviewed the information available on the potential toxicity of each of the citrus peel oil–derived ingredients as a whole, complex substance; CIR did not review the potential toxicity information on the individual constituents of which the citrus-derived ingredients are comprised. Cosmetic Ingredient Review requested information on the concentrations (including ranges, means, upper 95% confidence limits, detection limits, etc) of individual constituents in the citrus peel oil–derived ingredients used in cosmetics, to facilitate the safety assessment of these ingredients. Such information on constituents that have been identified as of concern by the Panel in previous safety assessments, or by other recognized scientific expert review bodies, is especially important.

Citrus oils contain large amounts of monoterpene hydrocarbons; limonene is present in the greatest amount, composing 38.1% to 95.8% of the oils. Citrus oils also contain sesquiterpene hydrocarbons, which are responsible for the characteristic flavor of these oils.

*Citrus limon* (lemon) peel oil has the most reported uses in cosmetics and personal care products, with a total of 490; more than half of the uses are in leave-on skin care preparations. The range of highest maximum use concentrations for *C limon* (lemon) peel oil is 0.0001% to 0.5%, with 0.5% reported in "other" skin care preparations. *Citrus aurantium dulcis* (orange) peel oil (reported as *C sinensis* [sweet orange] peel oil to the VCRP) has the second greatest number of overall uses reported, with a total of 289; about half of those uses are in leave-on skin care preparations. *Citrus aurantium dulcis* (orange) peel oil had a highest maximum use concentration range of 0.00002% to 29%, with 29% reported in noncoloring hair conditioners.

Under the rules governing cosmetic products in the European Union, citrus-derived ingredients must have furocoumarin content below 1 mg/kg in sun-protection and bronzing products. International Fragrance Association also has issued standards for citrus oils and other furocoumarin-containing essential oils. Finished products that are applied to the skin, excluding rinse-off products like bath preparations and soaps, must not contain more than 0.0015% or 15 ppm 5-MOP. If the level of 5-MOP has not been determined, limits specified for individual oils should be observed, and when such oils are used in combination with other phototoxic ingredients, the potential additive effect should be taken into consideration and use levels should be reduced accordingly. Restrictions for furocoumarin-containing essential oils and limits on the amounts of citrus-derived oils in finished products have been recommended for bitter orange oil expressed, grapefruit oil expressed, lemon oil cold-pressed, and lime oil expressed.

The European Union also set standards for limonene, stating that limonene must be included in the list of ingredients when its concentration exceeds 0.001% in leave-on products and 0.01% in rinse-off products.

The citrus peel oils in this assessment are found in foods, and the daily exposure from food use would result in a much larger systemic dose than that resulting from use in cosmetic products. Essential oils, oleoresins (solvent-free), and natural extractives (including distillates) derived from some citrus fruits are GRAS for their intended use in foods for human and animal consumption.

The dermal $LD_{50}$ of undiluted mandarin peel oil (*C reticulata*) and undiluted sweet orange oil was greater than 5 g/kg in rabbits. In undiluted lemon oil, the dermal $LD_{50}$ was greater than 10 g/kg in rabbits.

No genotoxic effects were observed in lemon oil or sweet orange oil in bacterial reverse mutation assays, mouse lymphoma cell mutation assays, and Chinese hamster chromosome aberration assays. Tumor-promoting activity was observed in mouse skin exposed to undiluted essential oils of orange (sweet), lemon, grapefruit, or lime after pretreatment with DMBA. Related studies of 40%, 80%, or 100% orange oil following pretreatment with DMBA or urethane also reported tumor-promoting activity, although the effect was weaker in the mice induced with urethane. No tumorigenic effects were observed in mice tested with orange oil without pretreatment with DMBA or urethane. Survival rates of the mice, including controls, in these experiments were poor because of a very high incidence of renal disease.

Irritation was observed in animals treated with unspecified concentrations of mandarin peel oil. In human patients, no irritation was observed after topical exposure to lemon oil (up to 20%) or mandarin peel oil (8%).

In rabbits, lemon oil tested at 5% was not irritating and orange oil tested undiluted did not induce significant or irreversible damage to the eyes. Mandarin peel oil (8% in petrolatum) was not sensitizing in human maximization tests. In studies of 250 patients with dermatitis, less than 2.5% had positive reactions to bitter orange oil, lemon oil, or sweet orange oil tested at 2% in paraffin.

Phototoxic potential was observed for sweet orange oil and lemon oil in in vitro studies. No phototoxic responses were noted in some animal studies of lime oil, lemon oil, grapefruit oil, mandarin oil, or tangerine oil; however, phototoxic reactions were observed in response to undiluted lime oil and lime oil diluted to concentrations of 15%, undiluted bitter orange oil, and undiluted lemon oil and lemon oil diluted to concentrations of 50%. In human studies, phototoxic reactions were observed in response to undiluted bitter orange oil, lemon oil (1%), sweet orange oil (1%), and undiluted and diluted expressed lime oil (30%). Many of the citrus-derived peel oils contain constituents that are photoactive agents, although those noted to be furocoumarin free tended not to induce photosensitization.

A retrospective occupational study of food handlers noted positive reactions to orange and lemon peels. No published studies on reproductive and development toxicity of citrus-derived peel oils were discovered and no unpublished data were submitted to address these topics.

## Discussion

The citrus ingredients in this assessment are found in foods, and daily exposures from the consumption of foods can be expected to yield much larger systemic exposures to these ingredients than those from the use of cosmetic products. Essential oils, oleoresins (solvent-free), and natural extracts (including distillates) derived from some citrus fruits are GRAS in foods and animal feeds. Additionally, volatile oils of limes, lemons, grapefruits, bitter oranges, oranges, and tangerines are used as flavoring agents. Consequently, the primary focus of this safety assessment is on the potential for irritation and sensitization from dermal exposures to the citrus ingredients.

Most of the reports that the Panel reviewed were not sufficiently detailed to enable determining how well the substances tested represent the cosmetic ingredients included in this safety assessment. For example, the genus and species of the plants from which the test substances were derived, the cultivation methods used to grow the plants, and the methods of extraction are not specified in many of these studies.

The Panel expressed concern about the potential for constituents in citrus-derived peel oils, including the furocoumarin 5-MOP, to cause phototoxicity. International Fragrance Association has issued standards for citrus oils and other furocoumarin-containing essential oils, and the Panel agreed that adherence to the IFRA standards for such constituents will prevent phototoxicity. According to these standards, finished products that are applied to the skin, excluding rinse-off products, must not contain more than 0.0015%, or 15 ppm, 5-MOP. An IFRA standard also has been issued for 7-methoxycoumarin; based on established maximum levels of this substance from commercially available natural sources (like essential oils, extracts, and absolutes), exposure to 7-methoxycoumarin from the use of these oils and extracts is regarded to be acceptable if the level of 7-methoxycoumarin in the finished product does not exceed 100 ppm.

Additionally, based on the findings of a rodent carcinogenicity study in which tumor promotion activity may have been caused by repeated skin irritation and resultant proliferation of DMBA-treated basal cells, the Panel concluded that citrus-derived peel oils could potentially act as tumor promoters if formulated to reach irritant levels. Thus, these botanical ingredients must be formulated to be nonirritating.

The Panel noted that, because botanical ingredients are complex mixtures, there is concern that multiple botanical ingredients may each contribute to the final concentration of a single constituent. Therefore, when formulating products, manufacturers should avoid reaching levels in final formulation of plant constituents that may cause sensitization or other adverse effects. Specific examples of constituents that could induce adverse effects are limonene, citral, and other monoterpenes, furocoumarins (such as 5-MOP and 7-methoxycoumarin).

Finally, the Panel expressed concern about pesticide residues and heavy metals that may be present in botanical ingredients. They stressed that the cosmetics industry should continue to use current good manufacturing practices to limit impurities.

## Conclusion

The CIR Expert Panel concluded the citrus-derived peel oils are safe for use in cosmetic products, excluding rinse-off products, do not contain more than 0.0015% (15 ppm) 5-MOP, and when formulated to be nonsensitizing and nonirritating.

*C aurantifolia* (lime) peel oil*
*Citrus aurantium* amara (bitter orange) peel oil
*Citrus aurantium currassuviensis* peel oil*
*Citrus aurantium dulcis* (orange) peel oil
*Citrus clementina* peel oil*
*Citrus grandis* (grapefruit) peel oil
*Citrus iyo* peel oil*
*Citrus junos* peel oil
*Citrus limon* (lemon) peel oil
*Citrus medica vulgaris* peel oil*
*Citrus nobilis* (mandarin orange) peel oil
*Citrus reticulata* (tangerine) peel oil*
*Citrus tachibana/reticulata* peel oil*
*Citrus tangerina* (tangerine) peel oil *

*Not reported to be in current use. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in this group.

### Author's Note

Unpublished sources cited in this report are available from the Executive Director, Cosmetic Ingredient Review, 1620 L Street, NW, Suite 1200, Washington, DC 20036, USA.

### Author Contributions

Burnett, C. contributed to conception and design; acquisition, analysis, and interpretation; drafted manuscript; and critically revised manuscript. Fiume, M., Gill, L., and Heldreth, B. contributed to analysis and interpretation and critically revised manuscript. Bergfeld, W., Belsito, D., Hill, R., Klaassen, C., Liebler, D., Marks, J. Shank, R., Slaga, T., and Snyder, P. contributed to conception and design; analysis and interpretation; and critically revised manuscript; and gave final approval. All authors gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

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# SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006

Version 6.4
Revision Date 09.09.2021
Print Date 23.11.2021
GENERIC EU MSDS - NO COUNTRY SPECIFIC DATA - NO OEL DATA

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

### 1.1 Product identifiers

| | | |
|---|---|---|
| Product name | : | Lemon oil |
| Product Number | : | W262528 |
| 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. | : | 8008-56-8 |

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

| | | |
|---|---|---|
| Identified uses | : | Laboratory chemicals, Manufacture of substances |

### 1.3 Details of the supplier of the safety data sheet

| | | |
|---|---|---|
| Company | : | Merck Life Science Sp.z.o.o.<br>Szelągowska 30<br>PL-61-626 POZNAN |
| Telephone | : | +48 61 8290-100 |
| Fax | : | +48 61 8290-120 |
| E-mail address | : | TechnicalService@merckgroup.com |

### 1.4 Emergency telephone

| | | |
|---|---|---|
| Emergency Phone # | : | +(48)-223988029 (CHEMTREC)<br>998 (Straz pozarna) |

## 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
Skin irritation (Category 2), H315
Skin sensitization (Category 1), H317
Aspiration hazard (Category 1), H304
Short-term (acute) aquatic hazard (Category 1), H400
Long-term (chronic) aquatic hazard (Category 1), H410

For the full text of the H-Statements mentioned in this Section, see Section 16.



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## 2.2 Label elements

**Labelling according Regulation (EC) No 1272/2008**

Pictogram

| | |
|---|---|
| Signal word | Danger |
| Hazard statement(s) | |
| H226 | Flammable liquid and vapor. |
| H304 | May be fatal if swallowed and enters airways. |
| H315 | Causes skin irritation. |
| H317 | May cause an allergic skin reaction. |
| H410 | Very toxic to aquatic life with long lasting effects. |
| Precautionary statement(s) | |
| P210 | Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. |
| P273 | Avoid release to the environment. |
| P280 | Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection. |
| P301 + P310 | IF SWALLOWED: Immediately call a POISON CENTER/ doctor. |
| P303 + P361 + P353 | IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. |
| P331 | Do NOT induce vomiting. |
| Supplemental Hazard Statements | none |

**Reduced Labeling (<= 125 ml)**

Pictogram

| | |
|---|---|
| Signal word | Danger |
| Hazard statement(s) | |
| H317 | May cause an allergic skin reaction. |
| H304 | May be fatal if swallowed and enters airways. |
| Precautionary statement(s) | |
| P301 + P310 | IF SWALLOWED: Immediately call a POISON CENTER/ doctor. |
| P331 | Do NOT induce vomiting. |
| 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

CAS-No. : 8008-56-8

| Component | Classification | Concentration |
|---|---|---|



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| Lemon oil | | | |
|---|---|---|---|
| CAS-No. | 8008-56-8 | Flam. Liq. 3; Skin Irrit. 2; Skin Sens. 1; Asp. Tox. 1; Aquatic Acute 1; Aquatic Chronic 1; H226, H315, H317, H304, H400, H410 | <= 100 % |

For the full text of the H-Statements mentioned in this Section, see Section 16.

## SECTION 4: First aid measures

### 4.1 Description of first-aid measures

**General advice**
Show this material safety data sheet to the doctor in attendance.

**If inhaled**
After inhalation: fresh air.

**In case of skin contact**
In case of skin contact: Take off immediately all contaminated clothing. Rinse skin with water/ shower. Consult a physician.

**In case of eye contact**
After eye contact: rinse out with plenty of water. Remove contact lenses.

**If swallowed**
After swallowing: caution if victim vomits. Risk of aspiration! Keep airways free. Pulmonary failure possible after aspiration of vomit. Call a physician immediately.

### 4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

### 4.3 Indication of any immediate medical attention and special treatment needed

No data available

## SECTION 5: Firefighting measures

### 5.1 Extinguishing media

**Suitable extinguishing media**
Dry powder Dry sand

**Unsuitable extinguishing media**
Do NOT use water jet.

### 5.2 Special hazards arising from the substance or mixture

Nature of decomposition products not known.
Combustible.
Vapors are heavier than air and may spread along floors.
Forms explosive mixtures with air at elevated temperatures.
Development of hazardous combustion gases or vapours possible in the event of fire.

### 5.3 Advice for firefighters

Stay in danger area only with self-contained breathing apparatus. Prevent skin contact by keeping a safe distance or by wearing suitable protective clothing.



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### 5.4 Further information

Remove container from danger zone and cool with water. Prevent fire extinguishing water from contaminating surface water or the ground water system.

## SECTION 6: Accidental release measures

### 6.1 Personal precautions, protective equipment and emergency procedures

Advice for non-emergency personnel: Do not breathe vapors, aerosols. Avoid substance contact. Ensure adequate ventilation. Keep away from heat and sources of ignition.
Evacuate the danger area, observe emergency procedures, consult an expert.
For personal protection see section 8.

### 6.2 Environmental precautions

Do not let product enter drains. Risk of explosion.

### 6.3 Methods and materials for containment and cleaning up

Cover drains. Collect, bind, and pump off spills. Observe possible material restrictions (see sections 7 and 10). Take up carefully with liquid-absorbent material (e.g. Chemizorb®). Dispose of properly. Clean up affected area.

### 6.4 Reference to other sections

For disposal see section 13.

## SECTION 7: Handling and storage

### 7.1 Precautions for safe handling

**Advice on protection against fire and explosion**
Keep away from open flames, hot surfaces and sources of ignition.Take precautionary measures against static discharge.

**Hygiene measures**
Immediately change contaminated clothing. Apply preventive skin protection. Wash hands and face after working with substance.
For precautions see section 2.2.

### 7.2 Conditions for safe storage, including any incompatibilities

**Storage conditions**
Keep container tightly closed in a dry and well-ventilated place. Keep away from heat and sources of ignition.

**Storage class**
Storage class (TRGS 510): 3: Flammable liquids

### 7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated



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## SECTION 8: Exposure controls/personal protection

### 8.1 Control parameters

**Ingredients with workplace control parameters**

### 8.2 Exposure controls

**Personal protective equipment**

**Eye/face protection**
Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU). Safety glasses

**Skin protection**
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.

**Body Protection**
Flame retardant antistatic protective clothing.

**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**
Do not let product enter drains. Risk of explosion.

## SECTION 9: Physical and chemical properties

### 9.1 Information on basic physical and chemical properties

| | | |
|---|---|---|
| a) | Appearance | Form: liquid<br>Color: light yellow |
| b) | Odor | No data available |
| c) | Odor Threshold | No data available |
| d) | pH | No data available |
| e) | Melting point/freezing point | No data available |
| f) | Initial boiling point and boiling range | 176 °C - lit. |
| g) | Flash point | 57 °C - closed cup |
| h) | Evaporation rate | No data available |



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| | | |
|---|---|---|
| i) | Flammability (solid, gas) | No data available |
| j) | Upper/lower flammability or explosive limits | No data available |
| k) | Vapor pressure | No data available |
| l) | Vapor density | No data available |
| m) | Density | 0,853 g/cm3 at 25 °C |
| | Relative density | No data available |
| n) | Water solubility | slightly soluble |
| o) | Partition coefficient: n-octanol/water | No data available |
| p) | Autoignition temperature | No data available |
| q) | Decomposition temperature | No data available |
| r) | Viscosity | Viscosity, kinematic: No data available<br>Viscosity, dynamic: No data available |
| s) | Explosive properties | No data available |
| t) | Oxidizing properties | none |

**9.2 Other safety information**
No data available

## SECTION 10: Stability and reactivity

**10.1 Reactivity**
Vapor/air-mixtures are explosive at intense warming.

**10.2 Chemical stability**
The product is chemically stable under standard ambient conditions (room temperature) .

**10.3 Possibility of hazardous reactions**
No data available

**10.4 Conditions to avoid**
Heat, flames and sparks.
Heating.

**10.5 Incompatible materials**
Strong oxidizing agents

**10.6 Hazardous decomposition products**
In the event of fire: see section 5



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## SECTION 11: Toxicological information

### 11.1 Information on toxicological effects

**Acute toxicity**
LD50 Oral - Rat - 2.840 mg/kg
Remarks: (RTECS)
Inhalation: No data available
LD50 Dermal - Rabbit - > 5.000 mg/kg

**Skin corrosion/irritation**
Skin - Rabbit
Result: Mild skin irritation
(Draize Test)

**Serious eye damage/eye irritation**
No data available

**Respiratory or skin sensitization**
Local lymph node assay (LLNA) - Mouse
Result: positive
(OECD Test Guideline 429)

**Germ cell mutagenicity**
Test Type: Mouse
Test system: mouse lymphoma cells
Metabolic activation: with and without metabolic activation
Method: OECD Test Guideline 476
Result: negative

**Carcinogenicity**
No data available

**Reproductive toxicity**
No data available

**Specific target organ toxicity - single exposure**
No data available

**Specific target organ toxicity - repeated exposure**
No data available

**Aspiration hazard**
May be fatal if swallowed and enters airways.

### 11.2 Additional Information

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
Biodegradability Result: - Readily biodegradable.
No data available



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### 12.3 Bioaccumulative potential
No data available

### 12.4 Mobility in soil
No data available

### 12.5 Results of PBT and vPvB assessment
This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

### 12.6 Other adverse effects
No data available

## SECTION 13: Disposal considerations

### 13.1 Waste treatment methods

**Product**
See www.retrologistik.com for processes regarding the return of chemicals and containers, or contact us there if you have further questions.

## SECTION 14: Transport information

### 14.1 UN number
ADR/RID: 1993 IMDG: 1993 IATA: 1993

### 14.2 UN proper shipping name
ADR/RID: FLAMMABLE LIQUID, N.O.S. (Lemon oil)
IMDG: FLAMMABLE LIQUID, N.O.S. (Lemon oil)
IATA: Flammable liquid, n.o.s. (Lemon oil)

### 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 material safety data sheet complies with the requirements of Regulation (EC) No. 1907/2006.

**National legislation**

| | |
|---|---|
| Seveso III: Directive 2012/18/EU of the European Parliament and of the Council on the control of major-accident hazards involving dangerous substances. | : ENVIRONMENTAL HAZARDS |



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**Other regulations**
Take note of Dir 94/33/EC on the protection of young people at work.

**15.2 Chemical Safety Assessment**
For this product a chemical safety assessment was not carried out

## SECTION 16: Other information

**Full text of H-Statements referred to under sections 2 and 3.**

| | |
|---|---|
| H226 | Flammable liquid and vapor. |
| H304 | May be fatal if swallowed and enters airways. |
| H315 | Causes skin irritation. |
| H317 | May cause an allergic skin reaction. |
| H400 | Very toxic to aquatic life. |
| H410 | Very toxic to aquatic life with long lasting effects. |

**Further information**
The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

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