



Toxicological profile for Spearmint oil

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

Curled mint oil; Mentha spicata oil; Spearmint terpenes; CCRIS 7499; EPA Pesticide Code 128800; FEMA No. 3032; HSDB 1919; Oil of crispmint; Oil of curled mint; Oil of spearmint; Spearmint oil; Spearmint, oil (Mentha spicata L.); Spearmint oil, native (Mentha spicata); Spearmint oil, native terpeneless (Mentha spicata); UNII-C3M81465G5; Mint oil; Oils, Mentha spicata; Oils, spearmint (ChemIDplus)

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

8008-79-5

1.7. Properties

1.7.1. Melting point

(°C): <25 (EPISuite, 2017); 9.86 (estimated) (EPISuite, 2017)

1.7.2. Boiling point

(°C): 228.5 (EPISuite, 2017)

1.7.3. Solubility

Slightly soluble in water (HSDB, 2003); soluble 1 vol in 1 vol of 80% ethanol at 20°C (HSDB, 2003); 1300-1310 mg/L at 25°C (BPDB, 2021; EPISuite, 2017)

1.7.4. pKa

No data available to us at this time.

1.7.5. Flashpoint

61.11°C (NZ EPA CCID); 92°C (EFSA, 2012)

1.7.6. Flammability limits (vol/vol%)

"Not explosive" (BPDB, 2021)

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

Volatile (HSDB, 2003); Moderately volatile or highly volatile (BPDB, 2021)

1.7.10. Vapor pressure

0.13 mm Hg at 25°C (EPISuite, 2017); 21328 mPa [0.16 mmHg] at 25°C (BPDB, 2021)

1.7.11. log Kow

2.71 (EPISuite, 2017); 2.4 at 20°C and pH 7 (BPDB, 2021)

2. General information

2.1. Exposure

Food: Yes (Burdock, 2010)

Cosmetics: Yes (CosIng)

Environment: Yes (HSDB, 2003)

Pharmaceuticals: Yes (Martindale, 1999)

Reported uses (ppm): (FEMA, 1994)

Food category	Usual	Max
Alcoholic beverages	116.30	154.30
Baked goods	1,054.00	1,318.00
Chewing gum	1,977.00	7,913.00
Condiments,relishes	50.00	250.00
Frozen dairy	47.50	130.40
Fruit juice	500.00	550.00
Gelatins, puddings	89.00	95.42
Hard candy	1,472.00	1,605.00
Jams, jellies	96.95	98.66

Nonalcoholic beverages	107.20	136.00
Seasonings, flavors	66,668.00	66,668.00
Soft candy	463.80	559.60
Sweet sauce	39.00	90.00

Burdock lists an individual's consumption of spearmint oil (CAS RN 8008-79-5) as being 0.3997mg/kg bw/day, and the FEMA PADI (possible average daily intake) as 227.612 mg.

As taken from Fenaroli's Handbook of Flavor and Ingredients. Sixth Edition. Burdock G.A (2010). CRC Press. ISBN 978-1-4200-9077-2

Natural Pollution Sources:

Herbaceous Plant...In North America, England, Germany, & Holland. The Most Frequently Cultivated Varieties Are M Trichoura Brig, M Crispata, & M Tenuis. The Plant Is From 25-75 Cm (10-30 In) High, With An Erect, Branched Stalk...Long, Lanceolate Leaves, & Flowers Clustered...In Spikes. /Spearmint/ [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. 469] **PEER REVIEWED**

Flowering tops of *Mentha spicata* L (*M viridis* L), Labiatae. [Budavari, S. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Whitehouse Station, NJ: Merck and Co., Inc., 1996., p. 1168] **PEER REVIEWED**

TRADITIONAL MEDICINE: Peppermint, spearmint, and their oils reportedly used in both western and eastern cultures as aromatic, stomachic, stimulant, antiseptic, local anesthetic, and antispasmodic in treating indigestion, nausea, sore throat, diarrhea, colds, headaches, toothaches, and cramps. ... /has/ been reported used in cancers. [Leung, A.Y., Foster, S. Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics. New York, NY. John Wiley & Sons, Inc. 1996., p. 370] **PEER REVIEWED**

Primary use in flavors for toothpaste, chewing gum, candy, and mouthwashes where it blends well with peppermint. [Kirk-Othmer Encyclopedia of Chemical Technology. 4th ed. Volumes 1: New York, NY. John Wiley and Sons, 1991-Present., p. V17 664] **PEER REVIEWED**

As taken from HSDB, 2003.

Mentha viridis leaf oil (CAS RN 84696-51-5 / 8008-79-5) is used as an astringent, fragrance and skin conditioning agent; *Mentha spicata* flower/leaf/stem oil (no CAS RN given) as a fragrance and skin conditioning agent; and *Mentha spicata* leaf/stem oil (no CAS RN given) as a fragrance and skin conditioning - miscellaneous agent in cosmetics in the EU.

As taken from CosIng (Cosmetic substances and ingredients database).

Spearmint oil (CAS RN 8008-79-5) is listed as an ingredient (at concentrations, where specified) in auto, inside the home (0.01-1% in "old" products), personal care (0.1-1%), "old" pesticide (2%) and pet care (<2%) products by the CPID.

A fungicide and plant growth regulator often used as a potato sprouting inhibitor (BPDB, 2021).

Anticipated average usual use levels and average maximum use levels of spearmint oil (*Mentha spicata* L.):

	Average usual(ppm)	Average maximum(ppm)

baked goods :	1055	1320
beverage(nonalcoholic) :	110	140
beverages(alcoholic) :	120	155
chewing gum :	8000	24000
condiments / relishes :	50	250
frozen dairy :	50	130
gelatins / puddings :	90	95
hard candy :	5000	10000
jams / jellies :	72	1900
seasonings / flavors :	66670	66670
soft candy :	5000	10000
sweet sauces :	40	90

Anticipated average usual use levels and average maximum use levels of Scotch spearmint oil (*Mentha cardiaca* L.):

	Average usual(ppm)	Average maximum (ppm)
chewing gum :	8000	24000
condiments / relishes :	50	250
frozen dairy :	50	130
hard candy :	5000	10000

jams / jellies :	100	200
seasonings / flavors :	1000	5000
soft candy :	5000	10000
sweet sauces :	40	90

Anticipated average usual use levels and average maximum use levels of Erospicata oil (*Mentha spicata* 'Erospicata') and curly mint oil (*Mentha spicata* var. *crispa*):

	Average usual(ppm)	Average maximum (ppm)
baked goods :	140	300
beverage(nonalcoholic) :	39	99
beverages(alcoholic) :	150	240
chewing gum :	8300	8300
confectionery froastings :	650	650
frozen dairy :	95	110
gelatins / puddings :	50	200
hard candy :	320	1200
meat products :	6	8
soft candy :	320	1200

As taken from Marnett LJ et al. 2013. Food Technology 67(8), 38-56.

"The mint oils (especially spearmint oil) are commonly used as fragrance components in toothpastes, mouthwashes, gargles, soaps, detergents, creams, lotions, and perfumes. Maximum use levels reported for spearmint and cornmint oils are 0.4–0.8%, respectively, in perfumes."

"Spearmint oil are extensively used in flavoring chewing gums, candies, and chocolates as well as in most other food products, including alcoholic (liqueurs, etc.) and non-alcoholic beverages,

frozen dairy desserts, baked goods, gelatins and puddings, processed fruits, and sweet sauces. The highest average maximum use levels reported are About 0.132% (1318 ppm) for spearmint oil in baked goods.”

“Leaves (or oil) of spearmint, widely used as primary or adjunct flavoring for herb teas; capsules, tablets, tincture, and so on, in formulations for digestion, colds, and fevers”

“..... spearmint, and their oils reportedly used in both western and eastern cultures as aromatic, stomachic, stimulant, antiseptic, local anesthetic, and antispasmodic in treating indigestion, nausea, sore throat, diarrhea, colds, headaches, toothaches, and cramps”

As taken from Khan IA and Abourashed EA, 2010.

Spearmint oil, spearmint extract, spearmint absolute, spearmint oil, rectified, spearmint CO2 extract and spearmint oil terpenes (all CAS RN 8008-79-5) are reportedly used in fragrance compounds (IFRA), and spearmint oil (CAS RN 8008-79-5) is permitted for use in nonfood and fragrance use pesticide products (US EPA InertFinder database, 2022).

Spearmint oil (no CAS RN given) is used as a flavour enhancer in oral use non-medicinal natural health products and *Mentha viridis* (spearmint) leaf oil (no CAS RN given) is used as a cosmetic astringent, fragrance ingredient, and skin-conditioning agent in topical use non-medicinal natural health products (Health Canada, 2021).

2.2. Combustion products

As taken from Baker R and Bishop L. (2005). The pyrolysis of non-volatile tobacco ingredients using a system that simulates cigarette combustion conditions. J. Anal. Appl. Pyrolysis 74, 145–170.

Ingredient Name & CAS Number	Max. cig. appln. level (ppm)	Composition of pyrolysate (Compound, %)	Max. level in smoke (µg)
Spearmint oil 8008-79-5	21	Carvone (67.8)	7
		Limonene (8.3)	0.9
		beta-Bourbonene (2.5)	0.3
		Cineole (2.0)	0.2
		Caryophyllene + ? (1.8)	0.2

2.3. Ingredient(s) from which it originates

The volatile oil is obtained by steam distillation from the fresh overground parts of the flowering plant *mentha spicata*.

As taken from Burdock, 2010.

Source: Spearmint *Mentha spicata* L. (syn. *M. viridis* L.).

As taken from Khan IA and Abourashed EA, 2010.

Mentha viridis leaf oil (CAS RNs 84696-51-5 / 8008-79-5) is the volatile oil obtained from the dried tops and leaves of the garden mint (spearmint), *Mentha viridis* L., Labiatae.

Mentha spicata flower/leaf/stem oil is the volatile oil obtained from the flowers, leaves and stems of *Mentha spicata*, Lamiaceae.

Mentha spicata leaf/stem oil is the volatile oil obtained from the leaves and stems of Mentha spicata.

As taken from CosIng (Cosmetic substances and ingredients database).

CAS RN 8008-79-5 "TSCA Definition 2019: Extractives and their physically modified derivatives. Mentha spicata, Labiatae and occasionally Mentha cardiaca, Labiatae."

As taken from ChemIDplus

3. Status in legislation and other official guidance

Cosmetics	UK: Not listed in Schedule 1	Food	EU: yes, USA: GRAS; 182.20
Tobacco	Approved in Belgium, France, Germany and UK	ADI	Not identified
Listed by FEMA as generally recognised as safe (GRAS) for food flavouring use (FEMA, 1965)			

Plant oils / Spear mint oil (CAS RN 8008-79-5) is not registered under REACH (ECHA).

There is a REACH dossier on Essential oil of Spearmint obtained from the aerial part of Mentha spicata and/or Mentha cardiaca (Lamiaceae) obtained by distillation (no CAS RN given; EC no. 946-253-9) (ECHA).

Plant oils/ spear mint oil (CAS RN 8008-79-5), the Essential oil of Spearmint described above, spearmint oil natural (no CAS RN given; EC no. 932-413-5), and spearmint oil (no CAS RN given; EC no. 937-710-3) are not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2022).

In the USA, spearmint, oil (Mentha spicata L.) (CAS RN 8008-79-5) is included on the US FDA's inventory of "Substances Added to Food (formerly EAFUS)" as a flavoring agent or adjuvant, is generally recognized as safe (GRAS) under 21 CFR section 182.20 (Essential oils, oleoresins (solvent-free), and natural extractives (including distillates)), and is also covered under 21 CFR section 172.230 (Microcapsules for flavoring substances) (FDA, 2022a,b).

Spearmint oil (CAS RN 8008-79-5) is listed in the US EPA InertFinder Database (2022) as approved for non-food and fragrance use pesticide products.

Spearmint oil (CAS RN 8008-79-5) is exempt from the requirements of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) under the minimum risk exemption regulations in 40 CFR 152.25(f) (US EPA, 2015).

Spearmint oil (CAS RN 8008-79-5) 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.

Spearmint oil (CAS RN 8008-79-5) is reported to contain 67% l-carvone and 0.06% 1-octen-3-yl acetate which, as restricted constituents due to their sensitization potentials, must be taken into account when determining the maximum level that may be used in cosmetics (IFRA, 2020, 2022).

Spearmint oil, Mentha spicata L. (CAS RN 8008-79-5) has been given GRAS status by FEMA (Marnett et al. 2013), which has been affirmed by Cohen et al. (2020).

Oil of spearmint (CAS RN 8008-79-5) is listed by the US EPA Office of Pesticide Programs and was first registered as a "biochemical" pesticide on 6 November 1995.

Spearmint oil (CAS RN 8008-79-5) is included on the US FDA's list of inactive ingredients for approved drug products. It is permitted for use as an ingredient in various products, at the following maximum potencies per unit dose:

Inactive Ingredient	Route	Dosage Form	CAS Number	UNII	Maximum Potency per unit dose
SPEARMINT OIL	DENTAL	PASTE, DENTIFRICE	8008795	C3M81465G5	27.6%w/w
SPEARMINT OIL	ORAL	CAPSULE	8008795	C3M81465G5	NA
SPEARMINT OIL	ORAL	SOLUTION	8008795	C3M81465G5	NA
SPEARMINT OIL	ORAL	SUSPENSION	8008795	C3M81465G5	0.59mg/20ml
SPEARMINT OIL	ORAL	SYRUP	8008795	C3M81465G5	0.12mg/5ml
SPEARMINT OIL	SUBLINGUAL	TABLET	8008795	C3M81465G5	0.4mg
SPEARMINT OIL	TOPICAL	OINTMENT	8008795	C3M81465G5	NA

As taken from FDA, 2022c

"Acceptable daily intake" and acceptable operator exposure level for "huile de menthe verte" [oil of green mint] (no CAS RN given) are both reported to be 0.252 mg/kg bw/day [route of exposure not specified].

As taken from AGRITOX, 2020

Spearmint essential oil (no CAS RN given) is classified as a natural health product (NHP) under Schedule 1, item 2 (an extract) of the Natural Health Products Regulations (Health Canada, 2021).

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

No data available to us at this time.

4.2. Absorption, distribution and excretion

No data available to us at this time.

4.3. Interactions

"The effect of pretreatment with essential oils (EOs) from eight aromatic plants on the seizure latency and severity of pentylenetetrazol- (PTZ-) induced seizures in mice was evaluated. Weight-dependent doses of *Rosmarinus officinalis*, *Ocimum basilicum*, *Mentha spicata*, *Mentha pulegium*, *Lavandula angustifolia*, *Mentha piperita*, *Origanum dictamnus*, and *Origanum vulgare*, isolated from

the respective aromatic plants from NE Greece, were administered 60 minutes prior to intraperitoneal (i.p.) injection of a lethal dose of PTZ to eight respective groups of Balb-c mice. Control group received only one i.p. PTZ injection. Motor and behavioral activity of the animals after EOs administration, development of tonic-clonic seizures, seizure latency and severity, and percentage of survival after PTZ administration were determined for each group. All groups of mice treated with the EOs showed reduced activity and stability after the administration of the oil, except for those treated with *O. vulgare* (100% mortality after the administration of the oil). After PTZ administration, mice from the different groups showed increased latency and reduced severity of seizures (ranging from simple twitches to complete seizures). Mice who had received *M. piperita* demonstrated no seizures and 100% survival. The different drastic component and its concentration could account for the diversity of anticonvulsant effects.” As taken from Koutroumanidou E et al. 2013. *Epilepsy Res. Treat.* 2013, 532657. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23819045>

“Essential oils (EOs) can be used as alternative or complementary antifungal agents against human pathogenic moulds and yeasts. To reduce the effective dose of antimicrobial agents, EOs are combined which can lead to synergistic or additive effect. In this study the anti-yeast and anti-mould activities of selected EOs were investigated, alone and in combinations, against clinical isolates of *Candida albicans*, *C. parapsilosis*, *Aspergillus fumigatus*, *A. terreus*, *Rhizopus microsporus*, *Fusarium solani* and *Lichtheimia corymbifera*. Minimum inhibitory concentrations (MICs) were determined for the EOs of cinnamon, citronella, clove, spearmint and thyme. To investigate the combination effect of the EOs, fractional inhibitory concentrations (FICs) were defined by the checkerboard method and the type of interaction was determined by the FIC index (FICI). FIC index below 0.5 was considered as synergism and between 0.5 and 1 as additive effect. Strongest antifungal activity was showed by thyme EO with MIC values below 1.0 mg/ml. Combination of EOs resulted in additive or indifferent effect, with occasional "borderline synergism". The best combination was cinnamon with clove leading to additive effect in all cases.” As taken from Horváth G et al. 2016. *Acta Biol. Hung.* 67(2), 205-14. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/27165531>

“The aim of this study was to investigate the protective effect of *Mentha spicata* supplementation against nicotine-induced oxidative damage in the liver and erythrocytes of Wistar rats. Bioactive substances were determined by liquid chromatography - electrospray ionization - tandem mass spectrometry analysis. Animals were divided into 4 groups of 6 rats each: a normal control group, a nicotine-treated group (1 mg/kg), a group receiving *M. spicata* extract (100 mg/kg), and a group receiving both *M. spicata* extract (100 mg/kg) and nicotine (1 mg/kg). Many phenolic acids were identified in the *M. spicata* aqueous extract. After 2 months of treatment, nicotine induced an increase in the level of white blood cells and a marked decrease in erythrocytes, hemoglobin, and haematocrit. Aspartate transaminase, alanine transaminase, alkaline phosphatase, and lactate dehydrogenase activities were also found to be higher in nicotine-treated group than those of the control group. Furthermore, nicotine-treated rats exhibited oxidative stress, as evidenced by a decrease in antioxidant enzymes activities and an increase in lipid peroxidation level in liver and erythrocytes. Interestingly, the oral administration of *M. spicata* extract by nicotine-treated rats alleviated such disturbances. *M. spicata* contained bioactive compounds that possess important antioxidant potential and protected liver and erythrocytes against nicotine-induced damage.” As taken from Ben Saad A et al. 2018. *Appl. Physiol. Nutr. Metab.* 43(1), 77-83. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28892646>

5. Toxicity

5.1. Single dose toxicity

Rat oral LD50: 5 g/kg (Lewis 1996)

Rat, oral LD50 >2000 mg/kg bw
Rat, dermal LD50 >2000 mg/kg bw
Rat, inhalation LC50 >5.43 mg/L

As taken from EFSA, 2012.

Record for oils, spearmint (CAS RN 8008-79-5):

Chemical Purity	Species Scientific Name	Species Common Name	Exposure Type	Dose Number	Endpoint	Effect	Effect Measurement	Conc 1 (Author)	Conc 1 Units (Author)	Exposure/ Observed Duration (Days)	Observed Duration Units (Days)
>96	Mus musculus	House Mouse	Intraperitoneal	2	LOEL	Behavior	Sleeping time, induced	50	mg/kg	0.072	Day(s)

As taken from the US EPA ECOTOX database (2021).

Spearmint oil (CAS RN 8008-79-5):

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
LD50 - Lethal dose, 50 percent kill	Oral	Rodent - rat	5 gm/kg	Details of toxic effects not reported other than lethal dose value	FCTXAV Food and Cosmetics Toxicology. (London, UK) V.1-19, 1963-81. For publisher information, see FCTOD7. Volume(issue)/page/year: 16,871,1978

As taken from RTECS, 2019

5.2. Repeated dose toxicity

“Spearmint (*Mentha spicata* L.) and spearmint extracts are Generally Recognized as Safe (GRAS) for use as flavoring in beverages, pharmaceuticals, and confectionaries. Studies of spearmint extracts in humans and animals have reported conflicting results with respect to toxicity. Since the chemical composition of these extracts was not reported and the spearmint source material was different, the relevance of these existing data to evaluating the risks associated with ingestion of a dried aqueous spearmint extract standardized to rosmarinic acid is not clear. Hence, the safety and tolerability of the dried aqueous spearmint extract was evaluated as part of a double-blind, randomized, placebo-controlled trial in healthy adults with age-associated memory impairment. Ingestion of both 600 and 900 mg/day for 90 days had no effect on plasma levels of follicular stimulating hormone, luteinizing hormone, or thyroid stimulating hormone, or other safety parameters including vital signs, plasma chemistry or whole blood hematology values. Additionally, there were no reported severe adverse events, no significant between-group differences in the

number of subjects reporting adverse effects and the adverse events reported could not be attributed to ingestion of the extract. These results therefore show that ingestion of the aqueous dried spearmint extract is safe and well-tolerated” As taken from Lasrado JA et al. 2017. Regul. Toxicol. Pharmacol. 86, 167-176. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28286189>.

“This study was conducted to investigate the effects of high concentration of *Mentha spicata* on liver tissues. Fourteen mature male albino mice were used in this study and divided into two groups. Control group received 1 ml normal saline daily for 7 days through oro-gastric tube. The remained animals received 1ml of aqueous extract of *Mentha spicata* daily for 7 days through oro-gastric tube. At the end of experiment, all animals were dissected. Liver Samples were fixed in 10% buffered formalin and embedded in paraffin. Sections were prepared and stained with hematoxylin and eosin. The slides were examined macroscopically. The results showed that coagulative degeneration, degeneration of hepatocytes with necrosis, inflammation vacuolization, inflammatory cell infiltration and fatty degenerative changes as also hemorrhage and increased size of Kupffer cells and vacuolated cytoplasm. From this study it has been concluded that high concentration of aqueous extract of *Mentha spicata* have a side effect on the structure and function of liver tissue.” As taken from Ali LH. 2017. The Iraqi Journal of Agricultural Sciences 48(Special Issue), 68-11. Proquest, 2018 available at <https://search.proquest.com/openview/fc804392213993e329be22a06952e9d1/1?pq-origsite=gscholar&cbl=2045989>

Record for oils, spearmint (CAS RN 8008-79-5):

Chemical Purity	Species Scientific Name	Species Common Name	Exposure Type	Dose Number	Endpoint	Effect	Effect Measurement	Conc 1 (Author)	Conc 1 Units (Author)	Exposure/ Observed Duration (Days)	Observed Duration Units (Days)
>96	Mus musculus	House Mouse	Intraperitoneal	2	NR	Behavior	Sleeping time, induced	50	mg/kg	3	Day(s)

As taken from the US EPA ECOTOX database (2021).

5.3. Reproduction toxicity

“Purpose: Given the antiandrogenic effects of spearmint, in this study we evaluated the effects of its essential oil on polycystic ovarian syndrome in a rat model. Methods: Female rats were treated as follows: Control, normal rats which received 150 mg/kg spearmint oil or 300 mg/kg spearmint oil, or sesame oil; and PCOS-induced rats which received 150 mg/kg spearmint oil or 300 mg/kg spearmint oil, or sesame oil. Then the animals were killed and the levels of LH, FSH, testosterone and ovarian folliculogenesis were evaluated. Results: Spearmint oil reduced body weight, testosterone level, ovarian cysts and atretic follicles and increased Graafian follicles in PCOS rats. Conclusion: Spearmint has treatment potential on PCOS through inhibition of testosterone and restoration of follicular development in ovarian tissue.” As taken from Sadeghi Ataabadi M et al. 2017. Adv. Pharm. Bull. 7(4), 651-654. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/29399556>

5.4. Mutagenicity

In vivo

No chromosome damage (micronucleus test) was seen in bone marrow cells of male mice given a single intraperitoneal (ip) dose of up to 800 mg/kg bw or 4 ip doses, 24 hours apart, of 400 mg/kg bw and killed 24 hours after final dose (Hayashi et al. 1988).

In a test for mutation and recombination in *Drosophila melanogaster*, insects were exposed for 18 hours to the essential oil of *mentha spicata* (spearmint) placed on filter paper in the centre of a Petri dish. The test was positive for mutations and negative for recombinations (Franzios et al. 1997).

In vitro

In a chromosome aberration test, Chinese hamster lung (CHL) cells were exposed to "mint oil" continuously at up to 0.3 mg/ml until the cells were harvested 24 and 48 hours later or by pulse treatment at up to 0.5 mg/ml. The test was conducted without S9 for continuous treatment and with and without S9 for pulse treatment. No chromosome damage was detected (Sofuni, 1994). In a chromosome aberration test, Chinese hamster ovary (CHO) cells were treated with up to 0.125 mg/ml spearmint oil. Cells were harvested 24 hours after treatment. No damage was detected in this limited assay without metabolic activation (Ishidate et al. 1984).

"Mint oil" was tested at up to 0.1 µl/plate in an Ames bacterial mutation assay with and without S9 activation using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538. No mutagenic effect was detected (Sofuni, 1994).

A limited Ames assay with two *Salmonella typhimurium* strains, TA98 and TA100, with and without S9 activation, no mutagenic effects were seen with up to 1 mg spearmint oil/plate (Haresaku et al. 1985).

In an unreliable Ames test with a test material described as "spearmint essential oil" but actually prepared from spearmint fried in groundnut oil, at 5 or 10 µl [sic] per plate, mutagenic effects were reported in *Salmonella typhimurium* strains TA98, TA1535, TA1537 and TA1538 without S9 activation. However, very high background colony levels were found in this study and extremely low doses of "spearmint essential oil" were tested (Sivaswamy et al. 1991).

In an indicative test of DNA damage, the differential toxicity (rec) assay of "mint oil" at 15 mg/disc to *Bacillus subtilis* strains M45 and H17 was examined. A positive response was reported (Sofuni, 1994). In a similar study using 10 mg/disc a weak positive response was seen without S9 activation and a negative response with S9 (Anon, 1985).

Spearmint oil (CAS RN 8008-79-5):

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Reference
DNA repair		Bacteria - <i>Bacillus subtilis</i>	10 mg/disc	TOFOD5 Tokishikoroji Foramu. Toxicology Forum. (Saiensu Foramu, c/o Kida Bldg., 1-2-13 Yushima, Bunkyo-ku, Tokyo 113, Japan) V.6- 1983- Volume(issue)/page/year:

				8,91,1985

As taken from RTECS, 2019

5.5. Cytotoxicity

“The aim of the present study was to investigate chemical composition and antibacterial activity of essential oil from the leaf of *Mentha spicata* plant against common food-borne pathogenic bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Escherichia coli* O157:H7). Chemical composition of the essential oil was identified by gas chromatography coupled with mass spectrometer detector (GC-MS). The antibacterial activity of the essential oil was evaluated by broth microdilution method and agar disk diffusion assay. According to the result of GC-MS analysis, 18 components were identified, accounting for 99.89% of the whole essential oil. The main components were carvone (78.76%), limonene (11.50%), β -bourbonene (11.23%), cis-dihydrocarveol (1.43%), trans-caryophyllene (1.04%), menthone (1.01%), menthol (1%), and terpinen-4-ol (0.99). The essential oil exhibited moderate level of antibacterial activity against all test microorganisms. In general, Gram-positive bacteria were more susceptible to *M. spicata* essential oil than Gram-negative bacteria. *L. monocytogenes* was the most sensitive of the microorganisms to the antibacterial activity of *M. spicata* essential oil (inhibition zone = 22 mm and MIC and MBC = 2.5 μ L/mL). Based on our results, the essential oil of *M. spicata* plant collected from Kermanshah province, west of Iran, has a potential to be applied as antibacterial agent.” As taken from Shahbazi Y. 2015. J. Pathog. 2015, 916305. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26351584>

“The present study reports in vivo antifungal and antiaflatoxicogenic efficacy of *Mentha spicata* essential oil (EO) against toxigenic *Aspergillus flavus* strain LHP(C)-D6 in chickpea food system up to 12 months of storage. In addition, the mode of antifungal action of EO was also determined to understand the mechanism of fungal growth inhibition. The in vivo study with different concentrations of *M. spicata* EO showed dose-dependent decrease in fungal colony count as well as aflatoxin B1 concentration. The EO caused >50% protection in inoculated sets and >70% protection in uninoculated sets of chickpea food system against *A. flavus* at 1.0 μ L mL(-1) air concentration. However, at the same concentration, EO caused 100% inhibition to aflatoxin B1 production in both sets when analyzed through high-performance liquid chromatography (HPLC). The antifungal target of EO in fumigated cells of *A. flavus* was found to be the plasma membrane when analyzed through electron microscopic observations and ions leakage test. The EO fumigated chickpea seeds showed 100% seed germination and seedling growth after 12 months of storage. Based on these observations, *M. spicata* EO can be recommended as plant-based preservative for safe protection of food commodities during storage conditions against fungal and most importantly mycotoxin contaminations.” As taken from Kedia A et al. 2016. Protoplasma 253(3), 647-653. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/26338202>

“Chemical composition, antioxidant and anti-*Vibrio* spp. activities of the essential oil isolated from the aerial parts of *Mentha spicata* L. (spearmint) are investigated in the present study. The effect of the essential oil on *Vibrio* spp. biofilm inhibition and eradication was tested using the XTT assay. A total of 63 chemical constituents were identified in spearmint oil using GC/MS, constituting 99.9% of the total identified compounds. The main components were carvone (40.8% $\pm$ 1.23%) and limonene (20.8% $\pm$ 1.12%). The antimicrobial activity against 30 *Vibrio* spp. strains (16 species) was evaluated by disc diffusion and microdilution assays. All microorganisms were strongly affected, indicating an appreciable antimicrobial potential of the oil. Moreover, the investigated oil exhibited high antioxidant potency, as assessed by four different tests in comparison with BHT. The ability of the oil, belonging to the carvone chemotype, to inhibit or reduce *Vibrio* spp. biofilm warrants further investigation to explore the use of natural products in antibiofilm adhesion and reinforce the possibility of its use in the pharmaceutical or food industry as a natural antibiotic and seafood

preservative against *Vibrio* contamination.” As taken from Snoussi M et al. 2015. *Molecules* 20(8), 14402-24. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26262604>

“*Staphylococcus aureus* causes human infections and foodborne intoxications. This study explored the potential antibacterial actions of sage and spearmint essential oils (EOs) against both its planktonic and biofilm cells, in comparison with sodium hypochlorite (NaOCl), a commonly applied chemical sanitizer. Initially, the minimum inhibitory and bactericidal concentrations (MICs, MBCs) of each plant mixture were determined against planktonic cultures, following growth at 30°C for 24h. Stationary phase planktonic bacteria were then individually exposed for 6min to either each EO (applied at 1-2×MBC; 2.5-5%), or NaOCl (250-450ppm). These were also left to form biofilms on 96-well polystyrene microplates, at 30°C for 96h, with medium renewal at 48h, in the presence of 10 different concentrations of each EO, expanding from sub- to super-inhibitory for planktonic growth, and the minimum biofilm inhibitory concentrations (MBICs; >90% inhibition) of each plant mixture were calculated. Formed biofilms were finally exposed for 6min to either each EO (applied at 2-6×MBC; 5-15%), or NaOCl (7500-25,000ppm; applied either alone or in combination with each EO at 5%). Results showed that both EOs presented MIC and MBC equal to 1.25 and 2.5%, respectively. As expected, their application at their MIC and above significantly inhibited biofilm formation, while spearmint EO was still able to cause this at ½ of its MIC, with MBICs equal to 1.25 and 0.63% for sage and spearmint EOs, respectively. Alarming, the application of both EOs at 1/8 to 1/16 of their MIC further increased biofilm formation. Regarding biofilm disinfection experiments, the individual application of each EO against the pre-established sessile communities resulted in log decrease ranges of 0.8-3logCFU/cm², while in the case of NaOCl application (either alone or combined with each EO), the observed reductions never exceeded 1.7logCFU/cm². These last results highlight the great antimicrobial recalcitrance of biofilm communities, found here to be ca. 100 times more resistant to NaOCl compared to planktonic ones, and stress the urgent need for further research on alternative, adequate and safe disinfection strategies to control them in food processing and other environments.” As taken from Vetas D et al. 2017. *Int. J. Food Microbiol.* 257, 19-25. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/28633052>

“The study reports *Mentha cardiaca* essential oil (EO) as plant based preservative against fungal and aflatoxin contamination of stored dry fruits. Mycoflora analysis of the dry fruits revealed *Aspergillus favus* LHP-PV-1 as the most aflatoxigenic isolate with highest Aflatoxin B₁ content. *M. cardiaca* EO showed broad fungitoxic spectrum inhibiting the tested moulds contaminating dry fruits. Its minimum inhibitory concentration (MIC), minimum aflatoxin inhibitory concentration (MAIC) and minimum fungicidal concentration (MFC) against *A. favus* LHP-PV-1 were recorded to be 1.25, 1.0 and 2.25 µL/mL respectively. The EO caused decrease in ergosterol content and enhanced leakage of Ca²⁺, K⁺ and Mg²⁺ ions from treated fungal cells, depicting fungal plasma membrane as the site of antifungal action. The EO showed promising DPPH free radical scavenging activity (IC₅₀ value: 15.89 µL/mL) and favourable safety profile with LD₅₀ value (7133.70 mg/kg body wt.) when estimated through acute oral toxicity on mice. Carvone (61.62%) was recorded as the major component of the oil during chemical characterisation through GC-MS. Based on strong antifungal, antiaflatoxigenic and antioxidant potential, the chemically characterised *M. cardiaca* EO may be recommended as safe plant based preservative and shelf life enhancer of food items. This is the first report on antifungal and antiaflatoxigenic activity of *M. cardiaca* EO.” As taken from Dwivedy AK et al. 2017. *Food Chem. Toxicol.* 106(A), 175-184. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/28552513>

“*Ascosphaera apis* is one of the major fungal pathogens of honey bee broods and the causative agent of Chalkbrood disease. The factors responsible for the pathogenesis of Chalkbrood disease are still not fully understood, and the increasing resistance of *A. apis* to commonly used antifungal agents necessitates a search for new agents to control this disease. The in vitro antifungal activities of 27 plant essential oils against two isolates of *A. apis* (Aksu-4 and Aksu-9) were evaluated. Out of the 27 plant essential oils tested, 21 were found to be effective in killing both isolates of *A. apis*. Based on their minimum fungicidal concentration (MFC) values, the effective oils were grouped into three categories: highly effective, moderately effective and minimally effective. Mountain pepper oil,

Kala Bhangra oil, spearmint oil, babuna oil, betel leaf oil, carrot seed oil, cumin seed oil and clove bud oil were highly effective, with MBC values between 50.0 µg/mL and 600.0 µg/mL. Mountain pepper was the most effective essential oil, with an MBC value of 50.0 µg/mL. Citral and caryophyllene containing oils were the most effective with MIC 50 ppm. The essential oils tested exhibited significant antimicrobial activities against both strains of *A. apis*, and they may contain compounds that could play an important role in the treatment or prevention of Chalkbrood disease of honeybee." As taken from Ansari MJ et al. 2017. Saudi J. Biol. Sci. 24(5), 1001-1006. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/28663695>

"Natural products, known for their medicinal properties since antiquity, are continuously being studied for their biological properties. In the present study, we analyzed the composition of the volatile preparations of essential oils of the Greek plants *Ocimum basilicum* (sweet basil), *Mentha spicata* (spearmint), *Pimpinella anisum* (anise) and *Fortunella margarita* (kumquat). GC/MS analyses revealed that the major components in the essential oil fractions, were carvone (85.4%) in spearmint, methyl chavicol (74.9%) in sweet basil, trans-anethole (88.1%) in anise, and limonene (93.8%) in kumquat. We further explored their biological potential by studying their antimicrobial, antioxidant and antiproliferative activities. Only the essential oils from spearmint and sweet basil demonstrated cytotoxicity against common foodborne bacteria, while all preparations were active against the fungi *Saccharomyces cerevisiae* and *Aspergillus niger*. Antioxidant evaluation by DPPH and ABTS radical scavenging activity assays revealed a variable degree of antioxidant potency. Finally, their antiproliferative potential was tested against a panel of human cancer cell lines and evaluated by using the sulforhodamine B (SRB) assay. All essential oil preparations exhibited a variable degree of antiproliferative activity, depending on the cancer model used, with the most potent one being sweet basil against an in vitro model of human colon carcinoma." As taken from Fitsiou E et al. 2016. Molecules 21(8), E1069. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/27537869>

"Essential oils (EOs) can be used as alternative or complementary antifungal agents against human pathogenic moulds and yeasts. To reduce the effective dose of antimicrobial agents, EOs are combined which can lead to synergistic or additive effect. In this study the anti-yeast and anti-mould activities of selected EOs were investigated, alone and in combinations, against clinical isolates of *Candida albicans*, *C. parapsilosis*, *Aspergillus fumigatus*, *A. terreus*, *Rhizopus microsporus*, *Fusarium solani* and *Lichtheimia corymbifera*. Minimum inhibitory concentrations (MICs) were determined for the EOs of cinnamon, citronella, clove, spearmint and thyme. To investigate the combination effect of the EOs, fractional inhibitory concentrations (FICs) were defined by the checkerboard method and the type of interaction was determined by the FIC index (FICI). FIC index below 0.5 was considered as synergism and between 0.5 and 1 as additive effect. Strongest antifungal activity was showed by thyme EO with MIC values below 1.0 mg/ml. Combination of EOs resulted in additive or indifferent effect, with occasional "borderline synergism". The best combination was cinnamon with clove leading to additive effect in all cases." As taken from Horváth G et al. 2016. Acta Biol. Hung. 67(2), 205-14. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/27165531>

"CONTEXT: Mints (Lamiaceae) are used as traditional remedies for the treatment of several diseases. Their extracts are recognized as anti-inflammatory compounds. OBJECTIVE: This study characterized the cytotoxic effects of *Mentha spicata* L. (MS), *Mentha pulegium* L. (MP) and *Mentha rotundifolia* (L). Huds (MR) on macrophage cells (RAW264.7; U937) and determined their impact on apoptosis and autophagy, which can play a role in controlling inflammation. MATERIALS AND METHODS: The extracts were prepared in culture medium and tested from 25 to 400 µg/mL after 24-48 h of treatment. To show the effect of the aqueous ethanol (50%) extracts on apoptosis and autophagy, the presence of cleaved caspase-3, and the conversion of LC3-I to LC3-II was evaluated by Western blotting. RESULTS: Compared with the MTT assay, crystal violet showed a pronounced decrease in the number of cells with all extracts at 48 h. Calculated IC50 values were 257.31, 207.82 and 368.02 µg/mL for MS, MP and MR, respectively. A significant increase in PI positive cells was observed with all extracts at 200-400 µg/mL. Mitochondrial dysfunctions and

nuclear morphological changes were detected with MS and MR extracts at 400 µg/mL. At this concentration, no cleaved caspase-3 was found whereas stabilized caspase-3 in its dimeric form was identified. MS and MR extracts also favour LC3-I to LC3-II conversion which is a criterion of autophagy. CONCLUSIONS: The cytotoxic profiles depend on the extracts considered; MS extract showed the strong activity. However, all the mint extracts studied interact with the apoptotic and autophagic pathways at elevated concentrations.” As taken from Brahmi F et al. 2017. Pharm. Biol. 55(1), 286-293. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/27927076>

“Plant extracts and essential oils (EOs) are characterized by their antibacterial properties against various bacterial pathogens, including staphylococci. Some strains of these bacteria are resistant against the adverse effects of the environment including antibiotics, e. g. methicillin-resistant *Staphylococcus aureus* (MRSA). EOs alone cannot substitute for antibiotics but their treatment may be useful to intensify and strengthen the effects of antibiotics on pathogenic staphylococci. In this work, we tested the antibacterial effects of the essential oils of *Mentha* species with menthol as one of the effective substances against different strains of *S. aureus*. Two in vitro methods were used, the qualitative disc diffusion assay and the quantitative minimal inhibitory concentration (MIC) of selected essential oils. Peppermint oil from *Mentha piperita*, spearmint oil from *Mentha spicata* var. *crispa* and cornmint oil from *Mentha arvensis* were tested in this study against the various strains of *Staphylococcus aureus*, including methicillin resistant *Staphylococcus aureus* (MRSA). The oils were dissolved in DMSO (dimethyl sulfoxide) and diluted at the following ratios: 1 : 1, 1 : 2, 1 : 5, and 1 : 10. Based on the results determined by the agar disc diffusion test, the highest antibacterial properties were observed in spearmint oil against *S. aureus* CCM 4223 at 1 : 2 ratio where the inhibition zone varied at a range of 35.67 ± 6.81 mm. We determined also the MIC of all the oils where concentrations of the oils were as follows: 1 %; 0.5 %; 0.25 %; 0.125 % and 0.0625 %. The lowest concentrations of essential oils that possessed inhibitory effects on the growth of *S. aureus* varied between 0.125 % and 0.25 %.” As taken from Horvath P and Koscova J. 2017. Folia Veterinaria 61(3), 71-77. Available at <https://www.degruyter.com/view/j/fv.2017.61.issue-3/fv-2017-0030/fv-2017-0030.xml>

Spearmint oil (CAS RN 8008-79-5):

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
ICLo - Inhibitor Concentration Low	In vitro	Human - gastrointestinal tumor	250 mg/L/24H	In Vitro Toxicity Studies - other assays	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year:

As taken from RTECS, 2019

Mentha spicata L., ethanol extract (no CAS RN):

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
ICLo - Inhibitor Concentration Low	In vitro	Human - liver tumor	100 mg/L/48 H	In Vitro Toxicity Studies - cell proliferation:	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House,

				DNA incorporation , mitotic index etc.	Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/yea r: 86,16,2015

As taken from RTECS, 2017

“BACKGROUND: *Mentha spicata* (M. spicata) is a member of Lamiaceae that spreads mainly in the temperate and sub-temperate zones of the world. It is considered as a rich source of essential oils, which is widely used in pharmaceutical industries and food production. The objectives of the current study were to evaluate chemical composition, antioxidant, antimicrobial and antiproliferative activities associated with the essential oil of M. spicata cultivated in Algerian Saharan Atlas. METHODS: The aerial parts of M. spicata were subjected to hydrodistillation to produce the oil. Chemical identification of the oil composition was conducted by GC and GC-MS analyses. The antioxidant activity of the hydrodistilled oil was studied using DPPH, ABTS radical scavenging and ferric-reducing power assay. Antimicrobial potential was characterized against two microorganisms, signifying Gram positive, and Gram negative bacteria, and one *Candida* species. The microdilution method was employed to determine the minimum inhibitory concentration (MIC). The oil's antiproliferative effects against three human tumor cell lines were also investigated using the MTT assay, and the toxic doses that yielded 50% reduction of cell viability (LD50) were reported. RESULTS: Chemical analysis of the essential oil composition revealed 44 unique compounds with oxygenated monoterpenes (67.2%), followed by monoterpene hydrocarbons (20.8%), as the most abundant chemical components. Essential oil of M. spicata demonstrated moderate antioxidant activities as well as moderate to weak antimicrobial activities with best susceptibility observed for Gram positive bacteria towards the oil. In addition, anticancer activities that are associated with the oil against three human cancer cell lines were observed with LD50 values of 324 µg/mL, 279 µg/mL, 975 µg/mL against T47D, HCT-116 and MCF-7 cell lines, respectively. CONCLUSION: The results suggest that M. spicata essential oil may have potential value as a bioactive oil, for nutraceutical and medical applications, with its antioxidant, antimicrobial and antiproliferative activities.” As taken from Bardaweel SK et al. 2018. BMC Complement. Altern. Med. 18(1), 201. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/29970065>

“The study aimed to characterize the volatile constituents of *Mentha spicata* L., grown in Oman, and to investigate in vitro cytotoxic and antioxidant activities of the volatile oil. The pale-yellow color essential oil (% yield 0.55% v/w) showed the presence of thirteen compounds representing 99.19% of the total constituents. d-Carvone (65.21%) and dl-limonene (27.28%) were identified as the major volatile chemical constituents. Mint oil exhibited promising antioxidant (IC50 = 26.64 µg/mL) and cytotoxic activity (LC50 = 245 µg/mL). CLC predicted d-carvone to be active against thyroid gland undifferentiated (anaplastic) carcinoma, pancreatic carcinoma, brain carcinoma, and lymphoblastic leukemia while dl-limonene could be effective against skin melanoma, breast adenocarcinoma in addition to the above carcinomas. Omani spearmint volatile oil exhibited promising cytotoxic and antioxidant activities. However, further detailed studies are warranted to develop mint oil as a natural antioxidant and to unlock its hidden cytotoxic potential.” As taken from Alsaraf S et al. 2021. Biocatalysis and Agricultural Biotechnology 34, 102034. Available at <https://www.sciencedirect.com/science/article/abs/pii/S1878818121001304>

5.6. Carcinogenicity

Anticarcinogenicity

In an anticarcinogenicity study in the tumour-derived mouse cell line RAW264.7, a strong indications of anticarcinogenic action (inhibition of inducible nitric oxide synthase) was obtained with a methanol extract of "spearmint" (*Mentha vensis* L. var. *piperascens*) leaves at 200 µg/ml (Kim et al. 1998).

"Natural products, known for their medicinal properties since antiquity, are continuously being studied for their biological properties. In the present study, we analyzed the composition of the volatile preparations of essential oils of the Greek plants *Ocimum basilicum* (sweet basil), *Mentha spicata* (spearmint), *Pimpinella anisum* (anise) and *Fortunella margarita* (kumquat). GC/MS analyses revealed that the major components in the essential oil fractions, were carvone (85.4%) in spearmint, methyl chavicol (74.9%) in sweet basil, trans-anethole (88.1%) in anise, and limonene (93.8%) in kumquat. We further explored their biological potential by studying their antimicrobial, antioxidant and antiproliferative activities. Only the essential oils from spearmint and sweet basil demonstrated cytotoxicity against common foodborne bacteria, while all preparations were active against the fungi *Saccharomyces cerevisiae* and *Aspergillus niger*. Antioxidant evaluation by DPPH and ABTS radical scavenging activity assays revealed a variable degree of antioxidant potency. Finally, their antiproliferative potential was tested against a panel of human cancer cell lines and evaluated by using the sulforhodamine B (SRB) assay. All essential oil preparations exhibited a variable degree of antiproliferative activity, depending on the cancer model used, with the most potent one being sweet basil against an in vitro model of human colon carcinoma." As taken from Fitsiou E et al. 2016. *Molecules* 21(8), E1069. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/27537869>

Plant oils / spear mint oil (CAS RN 8008-79-5) are suspected carcinogens. The Toolbox profiler Carcinogenicity (genotox and nongenotox) alerts by ISS gives an alert for carcinogenicity.

As taken from ECHA, 2016

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

5.7. Irritation/immunotoxicity

Spearmint oil (CAS RN 8008-79-5):

Type of Test	Route of Exposure	Species Observed	Dose Data	Reaction Severity	Reference
Standard Draize test	Administration onto the skin	Rodent - rabbit	500 mg/24H	Moderate	FCTXAV Food and Cosmetics Toxicology. (London, UK) V.1-19, 1963-81. For publisher information, see FCTOD7. Volume(issue)/page/year: 16,871,1978
Standard Draize test	Administration onto the skin	Rodent - guinea pig	100%	Mild	FCTXAV Food and Cosmetics Toxicology. (London, UK) V.1-19, 1963-81. For publisher information, see FCTOD7. Volume(issue)/page/year:

					16,871,1978

As taken from RTECS, 2019.

A skin irritant ... [Lewis, R.J. Sax's Dangerous Properties of Industrial Materials. 9th ed. Volumes 1-3. New York, NY: Van Nostrand Reinhold, 1996., p. 3008] **PEER REVIEWED**

As taken from HSDB, 2003.

“May cause gastrointestinal tract irritation with nausea, vomiting and diarrhea.”

Spearmint oil is a “respiratory tract irritant”.

As taken from BPDB, 2021.

Skin and respiratory tract sensitization

Carvone: an overlooked contact allergen cross-reacting with sesquiterpene lactones? (Abstract). The terpene l-carvone is one of the main constituents of spearmint oil. The sensitizing potential of l-carvone has been considered low, but it has occasionally caused contact allergy in users of spearmint toothpaste and chewing gum. l-Carvone is also an oxidation product of d-limonene that occurs in solvents used increasingly in industry. We included l-carvone 5% pet. in the standard patch test series. In the 1st year, 541 patients were tested and 15 (2.77%) had positive, and 12 doubtful positive (?+) reactions to l-carvone. The strongest reactions were observed in 9 patients with concomitant Compositae sensitivity. The key clinical features and other contact allergies of the patients are presented. When re-testing with l-carvone in the same or lower concentrations, only 2 out of 8 patients had positive reactions. Possible reasons for this discrepancy are discussed in terms of cross-reactions, concomitant sensitization, excited skin syndrome, irritancy and facilitated immunological response. As taken from Paulsen E et al. 1993. Contact Dermatitis 29(3), 138-43.

Multicentre study of allergic contact cheilitis from toothpastes (Abstract). The present work reports the results of a multicentre study of toothpaste allergic contact cheilitis (TACC). The study examined 54 patients with eczematous lesions on the lips, the possible cause of which was suspected to be the use of toothpastes. Patch tests were conducted with a standard series, a specially-targeted series (toothpaste cheilitis series, TCS), and with suspected toothpaste(s). A stop-restart test (SRT) was carried out with these, together with a use test to identify possible alternative products. The TCS produced 17 positive reactions in 13 patients, the most frequent being to spearmint oil. Of the 54 patients, 5 displayed positive reactions only to the TCS. The patch tests with toothpaste produced positive reactions in 11/32 patients, the SRT a positive response in 10/12 cases. The diagnosis of TACC was confirmed in 15/54 patients. Alternative products were identified for 5 patients. In conclusion, the allergens most frequently responsible for TACC were the flavourings, and the additional series proved to be useful in many cases (together with patch tests with toothpastes and the SRT) for correct diagnosis and to initiate effective prevention. As taken from Francalanci S et al. 2000. Contact Dermatitis 43(4), 216-22.

"*Mentha spicata* L. var. *viridis* oil (MVO) is a potent antifungal agent, but its application in the topical treatment is limited due to its irritancy and volatility. It was aimed to develop more efficient, chitosan-incrusted MVO microspheres with reduced volatility and lesser irritancy and to dispense it in the form of ointment. Simple coacervation technique was employed to microencapsulate MVO in chitosan matrix. Morphological properties and polymer cross-linking were characterized by scanning electron microscopy and differential scanning calorimetry, respectively. Optimization was carried out on the basis of entrapment efficiency (EE) using response surface methodology. Well-designed microspheres having smooth surface and spherical shape were observed. EE (81.20%) of optimum batch (R21) was found at 1.62% w/v of cross-linker, 5.4:5 of MVO to chitosan ratio and at 1000 rpm. R21 showed $69.38 \pm 1.29\%$ in vitro MVO release in 12 h and 96.92% retention of MVO in microspheres even after 8 week. Ointments of PEG 4000 and PEG 400 comprising MVO (F1) and R21 (F2) were developed separately. F2 showed comparatively broader zone of growth inhibition (13.33 ± 1.76 - 18.67 ± 0.88 mm) and less irritancy (PII 0.5833, irritation barely perceptible) than that of F1. F2 was able to avoid the direct contact of mild irritant MVO with the skin and to reduce its rapid volatility. Controlled release of MVO helped in lengthening the duration of availability of MVO in agar media and hence improved its therapeutic efficacy. In conclusion, a stable and non-irritant formulation with improved therapeutic potential was developed." As taken from Mishra N et al. 2016. AAPS PharmSciTech 17(2), 482-492. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/26248538>

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, ylangylang, 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 fragrancemix 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>

"This work was aimed at correlating the chemotype of three *Mentha* species cultivated in Romania with an in vivo study of the anti-inflammatory and antinociceptive effects of essential oils. The selected species were *Mentha piperita* L. var. *pallens* (white peppermint), *Mentha spicata* L. subsp. *crispata* (spearmint), and *Mentha suaveolens* Ehrh. (pineapple mint). Qualitative and quantitative analysis of the essential oils isolated from the selected *Mentha* species was performed by gas chromatography coupled with mass spectrometry (GC-MS). The anti-inflammatory activity of the essential oils was determined by the rat paw edema test induced by λ -carrageenan. The antinociceptive effect of the essential oils was evaluated by the writhing test in mice, using 1% (v/v) acetic acid solution administered intraperitoneally and by the hot plate test in mice. The results showed a menthol chemotype for *M. piperita pallens*, a carvone chemotype for *M. spicata*, and a piperitenone oxide chemotype for *M. suaveolens*. The essential oil from *M. spicata* L. (EOMSP) produced statistically significant and dose-dependent anti-inflammatory and antinociceptive effects." As taken from Mogosan C et al. 2017. Molecules 22(2), E263. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/28208614>

Plant oils / spear mint oil (CAS RN 8008-79-5) are suspected skin sensitizers. The Toolbox profiler Protein binding alerts for skin sensitization by OASIS v1.3 gives an alert for skin sensitization. The CAESAR skin sensitization model in VEGA (Q)SAR platform predicts that the chemicals are sensitizers (good reliability).

As taken from ECHA, 2016

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

5.8. All other relevant types of toxicity

This ingredient was considered as part of an overall safety assessment of ingredients added to oral product (snus/nicotine pouch). Non-clinical in vitro studies were conducted to investigate the characteristics of extracts from tobacco free nicotine pouches alongside a reference snus product containing Spearmint Oil and/or 1R6F reference cigarette. Within the sensitivity and specificity of the bioassay(s) all smokeless products extracts were non-mutagenic, non-genotoxic, non-cytotoxic and non-irritant in contrast to results obtained for the 1R6F reference cigarette (Miller-Holt et al., 2022). Table below provides highest tested level(s) and specific endpoint(s).

Product type	Endpoint	Tested level (ppm)	Reference
Nicotine Pouch	In vitro genotoxicity	12000	Labstat International Inc. (2020)
	In vitro cytotoxicity	12000	Labstat International Inc. (2020)
	In vitro irritation (EpiGingival™ 3D)	12000	Charles River Laboratories (2022)

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

Endpoint	Tested level (ppm)	Reference
In vitro genotoxicity	994	JTI KB Study Report(s)
In vitro cytotoxicity	994	JTI KB Study Report(s)

Suppression of tumor necrosis factor-alpha-induced neutrophil adherence responses by essential oils (Abstract). In aromatherapy, essential oils are used as anti-inflammatory remedies, but experimental studies on their action mechanisms are very limited. AIMS: To assess their anti-inflammatory activities, effects of essential oils on neutrophil activation were examined in vitro. METHODS: Neutrophil activation was measured by tumor necrosis factor-alpha (TNF-alpha)-induced adherence reaction of human peripheral neutrophils. RESULTS: All essential oils tested at 0.1% concentration suppressed TNF-alpha-induced neutrophil adherence, and, in particular, lemongrass, geranium and spearmint oils clearly lowered the reaction even at 0.0125%. Similar inhibitory activities for the neutrophil adherence were obtained by their major constituent terpenoids: citral, geraniol, citronellol and carvone. In contrast, very popular essential oils, tea tree oil and lavender oil, did not display the inhibitory activity at the concentration. CONCLUSION: Thus, some essential oils used as antiinflammatory remedies suppress neutrophil activation by TNF-alpha at a low concentration (0.0125-0.025 %) in vitro. Abe S et al., Mediators Inflamm. 2003, Dec; 12(6):323-8

"In the present study, the medicinal aromatic plant *Mentha spicata* has been investigated as a source of essential oil (EO) and pharmaceuticals. The quantity and composition of EO from *M. spicata* cultivated in Palestine were analyzed seasonally over a three-year period. A significantly higher EO content was produced in summer and fall months (2.54-2.79%). Chemical analysis of EO revealed 31 compounds with oxygenated monoterpenes (90%) as the most abundant components followed by sesquiterpene and monoterpene hydrocarbons (6 and 3%, respectively). *M. spicata* can be characterized as a carvone chemotype (65%). EO and carvone have shown strong inhibitory activities against the principal enzymes associated with Alzheimer's disease (AD)

and overweight diseases (cholinesterase and porcine pancreatic lipase) and also shown strong antidermatophytic activity against *Microsporum canis*, *Trichophyton rubrum*, *T. mentagrophytes*, and *Epidermophyton floccosum*. The pancreatic lipase inhibition and the synergism showed the potential activity of *M. spicata* EO and carvone and that their combinations with standard drugs can be useful for the treatment of obesity and overweight. The results also demonstrated that, in addition to their significant inhibitory activity against biofilm formation of methicillin-resistant *Staphylococcus aureus* (MRSA), *M. spicata* EO and carvone had a strong inhibitory effect on metabolic activity and biomass of the preformed biofilm. The current study supports the utilization of *M. spicata* EO as a traditional medicine and opens perceptions to find more potent substances in the EO for the management of obesity, AD, and dermatophytosis and for combating drug-resistant bacterial infections." As taken from Ali-Shtayeh MS et al. 2019. Evid. Based Complement. Alternat. Med. 3834265. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31772594/>

6. Functional effects on

6.1. Broncho/pulmonary system

After suffering asthma attacks following the use of a toothpaste containing spearmint oil, a woman challenged (rinsing the mouth for 30 s) with 2 ml of a 0.02% solution of spearmint oil in 50% alcohol exhibited an "immediate and significant" bronchial response (Subiza et al., 1992).

"OBJECTIVE: To investigate the effect of spearmint oil on emphysema-like changes and the expression of tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-9) in lipopolysaccharide (LPS) treated rats. METHOD: Emphysematous changes model was induced by intratracheal instillation of LPS once a week for up to 8 weeks in rats. Rats were divided into control, dexamethasone (0.3 mg x kg⁻¹), and spearmint oil (10, 30, 100 mg x kg⁻¹) groups. Each group was treated with saline, dexamethasone, and spearmint oil respectively for 4 weeks. Then total and different white blood cell counts in bronchoalveolar lavage fluid(BALF) were carried out. The pathologic changes of lung tissue such as alveolar structure, airway inflammation, and goblet cell metaplasia were observed by HE and AB-PAS staining. Expression of TNF- α , IL-1 β , TIMP-1 and MMP-9 were measured. RESULT: Both spearmint and dexamethasone decreased the destruction of pulmonary alveolus. The total and different white blood cell counts in BALF including neutrophil and lymphocyte of spearmint oil 100 mg x kg⁻¹ and dexamethasone group were significantly reduced, and the goblet cell metaplasia was also inhibited. Dexamethasone had inhibitory effect on the expression of TNF- α , IL-1 β , TIMP-1 and MMP-9. Spearmint oil 30, 100 mg x kg⁻¹ significantly reduced TNF- α and IL-1 β respectively. Spearmint oil 10, 30 and 100 mg x kg⁻¹ had no effect on the expression of TIMP-1, but could decrease the expression of MMP-9 significantly in lung tissues. CONCLUSION: Spearmint oil has protective effect on rats with emphysematous changes, since it improves alveolar destruction, pulmonary inflammation, and goblet cell metaplasia. The mechanism may include reducing TNF- α , IL-1 β content and inhibiting overexpression of matrix metalloproteinase-9 in lung tissues." As taken from Liu J et al. 2011. Zhongguo Zhong Yao Za Zhi 36(8), 1054-9. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/21809585?dopt=AbstractPlus>

"BACKGROUND: Recently, there has been an increased interest in the effects of essential oils on athletic performances and other physiological effects. This study aimed to assess the effects of Citrus sinensis flower and Mentha spicata leaves essential oils inhalation in two different groups of athlete male students on their exercise performance and lung function. METHODS: Twenty physical education students volunteered to participate in the study. The subjects were randomly assigned into two groups: Mentha spicata and Citrus sinensis (ten participants each). One group was nebulized by Citrus sinensis flower oil and the other by Mentha spicata leaves oil in a concentration of (0.02 ml/kg of body mass) which was mixed with 2 ml of normal saline for 5 min before a 1500 m running tests. Lung function tests were measured using a spirometer for each

student pre and post nebulization giving the same running distance pre and post oils inhalation. RESULTS: A lung function tests showed an improvement on the lung status for the students after inhaling of the oils. Interestingly, there was a significant increase in Forced Expiratory Volume in the first second and Forced Vital Capacity after inhalation for the both oils. Moreover significant reductions in the means of the running time were observed among these two groups. The normal spirometry results were 50 %, while after inhalation with *M. spicata* oil the ratio were 60 %. CONCLUSION: Our findings support the effectiveness of *M. spicata* and *C. sinensis* essential oils on the exercise performance and respiratory function parameters. However, our conclusion and generalisability of our results should be interpreted with caution due to small sample size and lack of control groups, randomization or masking. We recommend further investigations to explain the mechanism of actions for these two essential oils on exercise performance and respiratory parameters.” As taken from Jaradat NA et al. 2016. J. Int. Soc. Sports Nut. 13, 36. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/27688737>

6.2. Cardiovascular system

No data available to us at this time.

6.3. Nervous system

“Osteoarthritis, as the major cause of chronic musculoskeletal pain, impacts people aged 45 and above. The first line analgesic treatments have reported minimal short term effects. The use of essential oils as pain killer has increased, recently. *Mentha spicata*, or spearmint essential oil is famous due to its anti-flatulence effects, but one less known biological activity of spearmint is its analgesic activity. The aim of our study was to confirm the analgesic effects of *M. spicata* essential oil. In this review, we evaluated the articles on analgesic activities of *M. spicata* essential oil from different relevant databases (PubMed, Science Direct, Wiley, Taylor & Francis, and Springer) without limitation up to April 30, 2016. Different animal studies have reported the analgesic effects of *M. spicata* essential oil and its main abundant compounds such as carvone, limonene and menthol, also, the efficacy and safety of spearmint oil in reducing of pain severity were confirmed in osteoarthritis patients. In spite of the beneficial effects of spearmint oil in reducing of pain, other large clinical trials are required to confirm the efficacy and safety of *M. spicata* oil.” As taken from Mahboubi M. 2017. Complement. Ther. Clin. Pract. 26, 1-4. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/28107842>

“This work was aimed at correlating the chemotype of three *Mentha* species cultivated in Romania with an in vivo study of the anti-inflammatory and antinociceptive effects of essential oils. The selected species were *Mentha piperita* L. var. *pallenscens* (white peppermint), *Mentha spicata* L. subsp. *crispata* (spearmint), and *Mentha suaveolens* Ehrh. (pineapple mint). Qualitative and quantitative analysis of the essential oils isolated from the selected *Mentha* species was performed by gas chromatography coupled with mass spectrometry (GC-MS). The anti-inflammatory activity of the essential oils was determined by the rat paw edema test induced by λ -carrageenan. The antinociceptive effect of the essential oils was evaluated by the writhing test in mice, using 1% (v/v) acetic acid solution administered intraperitoneally and by the hot plate test in mice. The results showed a menthol chemotype for *M. piperita pallenscens*, a carvone chemotype for *M. spicata*, and a piperitenone oxide chemotype for *M. suaveolens*. The essential oil from *M. spicata* L. (EOMSP) produced statistically significant and dose-dependent anti-inflammatory and antinociceptive effects.” As taken from Mogosan C et al. 2017. Molecules 22(2), E263. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/28208614>

“Extracts of several members of the monoterpene-rich Lamiaceae sub-family Nepetoideae, including those from the *Salvia* (sage), *Melissa* (Lemon balm) and *Rosmarinus* (rosemary) genera, evince cognitive and mood effects in humans that are potentially related to their effects on cholinergic and GABAergic neurotransmission. To date, despite promising in vitro properties, the

cognitive and mood effects of the closely related *Mentha spicata* (spearmint) and *Mentha piperita* (peppermint) remain unexplored. This study therefore assessed the human cognitive/mood effects of the *M. spicata/piperita* essential oil with the most promising, brain-relevant in vitro properties according to pre-trial in vitro screening. Design: Organic spearmint and peppermint (*Mentha spicata/piperita*) essential oils were pre-screened for neurotransmitter receptor binding and acetylcholinesterase (AChE) inhibition. In a double-blind, placebo-controlled, balanced cross-over study, 24 participants (mean age 25.2 years) consumed single doses of encapsulated placebo and 50 μ l and 100 μ l of the most promising essential oil (peppermint with nicotinic/GABAA receptor binding and AChE inhibitory properties, that increased calcium influx in a CAD cell neuronal model). Psychological functioning was assessed with mood scales and a range of standardised, cognitively demanding tasks pre-dose and at 1, 3 and 6 h post-dose. Results: The highest (100 μ L) dose of essential oil improved performance on the cognitively demanding Rapid Visual Information Processing task (RVIP) at 1 h and 3 h post-dose and both doses attenuated fatigue and improved performance of the Serial 3 s subtraction task at 3 h post-dose. Conclusion: Peppermint (*Mentha piperita*) essential oil with high levels of menthol/menthone and characteristic in vitro cholinergic inhibitory, calcium regulatory and GABAA/nicotinic receptor binding properties, beneficially modulated performance on demanding cognitive tasks and attenuated the increase in mental fatigue associated with extended cognitive task performance in healthy adults. Future investigations should consider investigating higher doses.” As taken from Kennedy D et al. 2018. *Nutrients* 10(8), E1029. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/30087294>

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

“BACKGROUND: This study is aimed at determining the efficacy of *Mentha spicata* (*M. spicata*) and *Mentha × piperita* (*M. × piperita*) in preventing chemotherapy-induced nausea and vomiting (CINV). METHODS: This was a randomised, double-blind clinical trial study. Prior to the study, patients were randomly assigned into four groups to receive *M. spicata* or *M. × piperita*. Statistical analysis included the χ^2 test, relative risk, and Student's t-test. Fifty courses were analysed for each group that met our eligibility criteria. The treatment and placebo groups applied essential oils of *M. spicata*, *M. × piperita*, or a placebo, while the control group continued with their previous antiemetic regimen. Patients or guardians recorded the number of emetic events, the intensity of nausea over 20 h of chemotherapy, as well as any possible adverse effects that occurred during this time. RESULTS: There was a significant reduction in the intensity and number of emetic events in the first 24 h with *M. spicata* and *M. × piperita* in both treatment groups ($p < 0.05$) when compared with the control and no adverse effects were reported. The cost of treatment was also reduced when essential oils were used. CONCLUSION: *M. spicata* or *M. × piperita* essential oils are safe and effective for antiemetic treatment in patients, as well as being cost effective.” As taken from Tayarani-Najaran Z et al. 2013. *Ecancermedicallscience* 7, 290. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23390455>

“May cause gastrointestinal tract irritation with nausea, vomiting and diarrhea.”

As taken from BPDB, 2021.

This study was conducted to evaluate the hypoglycemic, hypocholesterolemic and antioxidant properties of *Mentha spicata* (Labiatae) leaves aqueous extract (MSLA) in alloxan-induced diabetic rats. In this study, hyperglycemia was induced in male rats by intraperitoneal injection of alloxan monohydrate (150 mg/kg). The aqueous extract of *M. spicata* was orally administered at a dose of 300 mg/kg body weight to diabetic rats for 21 days and the effects were compared with glibenclamide (2 mg/kg). Fasting blood sugar (FBS), body weight, lipid profile and serum malondialdehyde (MDA) were monitored at 0, 7, 14 and 21 days after induction of diabetes. Total phenol contents (TP) and reducing power (RP) were also evaluated. TP and RP of aqueous extract were 2.763 ± 0.39 mg Galic acid/gr and 0.026 ± 0.001 EC50 mg/mL, respectively. The LD50 of the extract was found to be ~ 1500 mg/kg. The administration of *M. spicata* aqueous extract produced

a significant reduction ($P=0.01$) in FBS, total cholesterol, triglyceride, low density lipoprotein-cholesterol and MDA (101.83 ± 4.33 , 95.66 ± 4.75 , 89.83 ± 5.26 , 26.20 ± 5.10 mg/dl and 1.53 ± 0.61 μ mol/l, respectively) in diabetic rats. These effects were comparable with the effects of standard antidiabetic drug (glibenclamide). The results of the investigation indicated that *M. spicata* leaf aqueous extract possess hypoglycemic, hypocholesterolemic and antioxidant properties in diabetic rats. Therefore, this study suggest a promising use of it for treatment of diabetes. As taken from Bayani M. et al. Iranian Journal of Pharmaceutical Research (2017), 16 (Special Issue): 75-82. Available at http://ijpr.sbm.ac.ir/article_1998_96d31c6ca9ae7d97a4ed944cc7a45c24.pdf

In studies on *Mentha* species, different methods are practiced, different dissolvent and essential oils of plants are used and various pharmacologic effects are determined. In the related studies on species, it is found that *Mentha* species have significant effects such as analgesic, anti-inflammatory, antipyretic, DNA damage protecting activity, antioxidant, anti-androgenic, antimicrobial, cytotoxic, antiviral, anticancer, antiemetic, antibacterial, antiallergic, antiparasitic, sedative, anti-chlamydial, radioprotection, anti-cholinesterase, hepatoprotective, antispasmodic, acute toxicity effect, anti-mutagenic, cardiovascular effects and anti-tumour effects. As taken from Sevindik M (2018). Pharmacological Properties of *Mentha* Species. J. Tradit. Med. Clin. Natur. 7(1), 1000259. Available at <https://www.omicsonline.org/open-access/pharmacological-properties-of-mentha-species-2573-4555-1000259.pdf>

Objective: The purpose of this study was to investigate the effects of supplementation with a spearmint (*Mentha spicata* L.) extract, high in polyphenols including rosmarinic acid, on cognitive performance, sleep, and mood in individuals with age-associated memory impairment (AAMI).

Design: Subjects with AAMI (N = 90; 67% female; age = 59.4 ± 0.6 years) were randomly assigned (n = 30/group) to consume 900, 600, or 0 mg/day (two capsules, once daily) spearmint extract for 90 days, in this double-blind, placebo-controlled trial. Assessments were completed for cognition (days 0, 45, and 90), sleep (days 0 and 90), and mood (days 0 and 90) by using the Cognitive Drug Research (CDR) System™, Leeds Sleep Evaluation Questionnaire (LSEQ), and Profile of Mood States (POMS™), respectively.

Results: Quality of working memory and spatial working memory accuracy improved after supplementation with 900 mg/day spearmint extract by 15% ($p=0.0469$) and 9% ($p=0.0456$), respectively, versus placebo. Subjects consuming 900 mg/day spearmint extract reported improvement in their ability to fall asleep, relative to subjects consuming placebo ($p=0.0046$). Overall treatment effects were evident for vigor-activity ($p=0.0399$), total mood disturbance ($p=0.0374$), and alertness and behavior following wakefulness ($p=0.0415$), with trends observed for improvements after spearmint supplementation relative to placebo.

Conclusions: These results suggest that the distinct spearmint extract may be a beneficial nutritional intervention for cognitive health in older subjects with AAMI.

As taken from Herrlinger KA et al. (2018). Spearmint Extract Improves Working Memory in Men and Women with Age-Associated Memory Impairment. J. Altern. Complement. Med. 24(1), 37-47. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/29314866>

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 Spearmint oil 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 Spearmint oil. Table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
Smoke chemistry	936	Baker et al., 2004a
	13	JTI KB Study Report(s)
	130	
	51,800	Gaworski et al., 2011 & Coggins et al., 2011b
In vitro genotoxicity	936	Baker et al., 2004c
	13	Renne et al., 2006
	13	JTI KB Study Report(s)
	130	
	180	fGLH Study Report (2010)
	51,800	Gaworski et al., 2011 & Coggins et al., 2011b
In vitro cytotoxicity	936	Baker et al., 2004c
	13	JTI KB Study Report(s)
	130	
	180	fGLH Study Report (2010)
	51,800	Gaworski et al., 2011 & Coggins et al., 2011b
Inhalation study	936	Baker et al., 2004c
	13	Renne et al., 2006
	13	JTI KB Study Report(s)
	130	
	51,800	Gaworski et al., 2011 & Coggins et al., 2011b
Skin painting	19	Gaworski et al., 1999
	13	JTI KB Study Report(s)
	130	

9. Heated/vapor emissions toxicity

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

Endpoint	Tested level (ppm)	Reference
In vitro genotoxicity	994	JTI KB Study Report(s)
In vitro cytotoxicity	994	JTI KB Study Report(s)

“PURPOSE OF REVIEW: Vaping is gaining popularity in the USA, particularly among teens and young adults. While e-cigs are commonly represented as safer alternatives to tobacco cigarettes, little is known regarding the health effects of their short- or long-term use, especially in individuals with pre-existing respiratory diseases such as asthma. Flavored e-cig liquids (e-liquids) and e-cig aerosols contain airway irritants and toxicants that have been implicated in the pathogenesis and worsening of lung diseases. In this review, we will summarize existing data on potential health effects of components present in e-cig aerosols, such as propylene glycol, vegetable glycerin, nicotine, and flavorings, and discuss their relevance in the context of asthma. RECENT FINDINGS: Recent survey data indicate that adolescents with asthma had a higher prevalence of current e-cig use (12.4%) compared to their non-asthmatics peers (10.2%) and conveyed positive beliefs about tobacco products, especially e-cigs. Similarly, a study conducted among high school students from Ontario, Canada, indicated a greater likelihood of e-cig use in asthmatics as compared to their non-asthmatic peers. Availability of different flavorings is often cited as the main reason among youth/adolescents for trying e-cigs or switching from cigarettes to e-cigs. Occupational inhalation of some common food-safe flavoring agents is reported to cause occupational asthma and worsen asthmatic symptoms. Moreover, workplace inhalation exposures to the flavoring agent diacetyl have caused irreversible obstructive airway disease in healthy workers. Additionally, recent studies report that thermal decomposition of propylene glycol (PG) and vegetable glycerin (VG), the base constituents of e-liquids, produces reactive carbonyls, including acrolein, formaldehyde, and acetaldehyde, which have known respiratory toxicities. Furthermore, recent nicotine studies in rodents reveal that prenatal nicotine exposures lead to epigenetic reprogramming in the offspring, abnormal lung development, and multigenerational transmission of asthmatic-like symptoms. Comparisons of the toxicity and health effects of e-cigs and conventional cigarettes often focus on toxicants known to be present in cigarette smoke (CS) (i.e., formaldehyde, nitrosamines, etc.), as well as smoking-associated clinical endpoints, such as cancer, bronchitis, and chronic obstructive pulmonary disease (COPD). However, this approach disregards potential toxicity of components unique to flavored e-cigs, such as PG, VG, and the many different flavoring chemicals, which likely induce respiratory effects not usually observed in cigarette smokers” As taken from Clapp PW and Jaspers KI. 2017. Curr. Allergy Asthma Rep. 17(11), 79. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28983782>

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

Endpoint	Tested level	Reference
Aerosol chemistry	0.1078 mg/1.2 mL and 0.1348 mg/1.5 mL	Logic (2019a)
In vitro genotoxicity	0.1078 mg/1.2 mL and 0.1348 mg/1.5 mL	Logic (2019a)
In vitro cytotoxicity	0.1078 mg/1.2 mL and 0.1348 mg/1.5 mL	Logic (2019a)
In vivo genotoxicity	0.1078 mg/1.2 mL and 0.1348 mg/1.5 mL	Logic (2019a)
Inhalation study	0.1078 mg/1.2 mL and 0.1348 mg/1.5 mL	Logic (2019a)

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

Endpoint	Tested level	Reference
Aerosol chemistry	0.0183 mg/(tobacco portion; 310 mg)	Logic (2019b)
In vitro genotoxicity	0.0183 mg/(tobacco portion; 310 mg)	Logic (2019b)
In vitro cytotoxicity	0.0183 mg/(tobacco portion; 310 mg)	Logic (2019b)
In vivo genotoxicity	0.0183 mg/(tobacco portion; 310 mg)	Logic (2019b)
Inhalation study	0.0183 mg/(tobacco portion; 310 mg)	Logic (2019b)

10. Ecotoxicity

10.1. Environmental fate

EPISuite provides the following data:

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

Bond Method :	7.73E-005 atm-m3/mole (7.83E+000 Pa-m3/mole)
Group Method:	4.15E-007 atm-m3/mole
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:	HLC: 7.000E-005 atm-m3/mole (7.092E+000 Pa-m3/mole) VP: 0.13 mm Hg (source: MPBPVP) WS: 367 mg/L (source: WSKOWWIN)

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

Log Kow used:	2.71 (exp database)
Log Kaw used:	-2.500 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate):	5.210
Log Koa (experimental database):	None

Probability of Rapid Biodegradation (BIOWIN v4.10):

Biowin1 (Linear Model):	0.6829
Biowin2 (Non-Linear Model) :	0.6041
Biowin3 (Ultimate Survey Model):	2.8447 (weeks)
Biowin4 (Primary Survey Model) :	3.6088 (days-weeks)
Biowin5 (MITI Linear Model) :	0.4543
Biowin6 (MITI Non-Linear Model):	0.3753
Biowin7 (Anaerobic Linear Model):	-0.1361

Ready Biodegradability Prediction:	NO

Hydrocarbon Biodegradation (BioHCwin v1.01):

Structure incompatible with current estimation method!

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

Vapor pressure (liquid/subcooled):	13.7 Pa (0.103 mm Hg)
Log Koa (Koawin est):	5.210
Kp (particle/gas partition coef. (m ³ /ug)):	2.18E-007
Mackay model:	3.98E-008
Octanol/air (Koa) model:	

Fraction sorbed to airborne particulates (phi):

Junge-Pankow model:	7.89E-006
Mackay model:	1.75E-005
Octanol/air (Koa) model:	3.18E-006

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

Hydroxyl Radicals Reaction:

OVERALL OH Rate Constant =	141.1986 E-12 cm ³ /molecule-sec
Half-Life =	0.076 Days (12-hr day; 1.5E6 OH/cm ³)
Half-Life =	0.909 Hrs
Ozone Reaction:	

OVERALL Ozone Rate Constant =	8.593750 E-17 cm ³ /molecule-sec
Half-Life =	0.133 Days (at 7E11 mol/cm ³)
Half-Life =	3.200 Hrs
Fraction sorbed to airborne particulates (phi): 1.27E-005 (Junge-Pankow, Mackay avg) 3.18E-006 (Koa method) Note: the sorbed fraction may be resistant to atmospheric oxidation	

Soil Adsorption Coefficient (KOCWIN v2.00):

Koc :	136.2 L/kg (MCI method)
Log Koc:	2.134 (MCI method)
Koc :	416.6 L/kg (Kow method)
Log Koc:	2.620 (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: 7.73E-005 atm-m³/mole (estimated by Bon SAR Method)

Half-Life from Model River:	10.53 hours
Half-Life from Model Lake:	217.7 hours (9.07 days)

Removal In Wastewater Treatment:

Total removal:	7.56 percent

Total biodegradation:	0.11 percent
Total sludge adsorption:	3.65 percent
Total to Air:	3.80 percent

(using 10000 hr Bio P,A,S)

Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.176	1.16	1000
Water	25.4	360	1000
Soil	74.3	720	1000
Sediment	0.183	3.24e+003	0

Persistence Time: 396 hr

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that spearmint oils (CAS RN 8008-79-5) are of uncertain persistence in the environment.

Data accessed August 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 spearmint oils (CAS RN 8008-79-5) are not inherently toxic to aquatic organisms and are of low ecotoxicological concern.

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

Record for oils, spearmint:

Spec. Name	Sci. Common	Exp. Type	Media Type	Resp. Site	Endpoint	Trend	Effect	Conc
Spec. Name	Common	Chem. Anal.	Loc	Obs. Dur.	BCF	Eff%	Effect Meas.	(Standardized) Appl. Rate

			(Days)				
CAS #/Chemical: 8008795 - Oils, Spearmint							
Insects/Spiders							
Aedes aegypti	S	FW		LC50	INC	MOR	F 56080 ug/L
Yellow Fever Mosquito	U	LAB	1			MORT	
Anopheles stephensi	S	FW		LC50	INC	MOR	F 49710 (21960-78960) ug/L
Mosquito	U	LAB	1			MORT	
Culex quinquefasciatus	S	FW		LC50	INC	MOR	F 62620 (41410-83720) ug/L
Southern House Mosquito	U	LAB	1			MORT	
Aedes aegypti	S	FW		LC90	INC	MOR	F 110280 (86100-176890) ug/L
Yellow Fever Mosquito	U	LAB	1			MORT	
Anopheles stephensi	S	FW		LC90	INC	MOR	F 100990 (77300-170330) ug/L
Mosquito	U	LAB	1			MORT	
Culex quinquefasciatus	S	FW		LC90	INC	MOR	F 118700 (94440-181180) ug/L
Southern House Mosquito	U	LAB	1			MORT	

As taken from the EPA ECOTOX database.

ECOSAR version 1.11 provides the following aquatic toxicity data for CAS RN 8008-79-5:

Values used to Generate ECOSAR Profile:

Log Kow: 3.066 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 1310 (mg/L, PhysProp DB exp value)

ECOSAR v1.11 Class-specific Estimations

Vinyl/Allyl Ketones

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Vinyl/Allyl Ketones :	Fish	96-hr	LC50	23.479
Vinyl/Allyl Ketones :	Daphnid	48-hr	LC50	11.205
Vinyl/Allyl Ketones :	Green Algae	96-hr	EC50	8.170
Vinyl/Allyl Ketones :	Fish		ChV	4.466
Vinyl/Allyl Ketones :	Daphnid		ChV	2.148 !
Vinyl/Allyl Ketones :	Green Algae		ChV	4.128 !
Vinyl/Allyl Ketones :	Fish (SW)	96-hr	LC50	84.344
Vinyl/Allyl Ketones :	Mysid (SW)	96-hr	LC50	4.554
Vinyl/Allyl Ketones :	Fish (SW)		ChV	47.663
Vinyl/Allyl Ketones :	Mysid (SW)		ChV	0.369 !
Neutral Organic SAR :	Fish	96-hr	LC50	13.602
(Baseline Toxicity) :	Daphnid	48-hr	LC50	8.522
	Green Algae	96-hr	EC50	9.538
	Fish		ChV	1.493
	Daphnid		ChV	1.093
	Green Algae		ChV	3.110

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

“This study evaluated effects of dietary supplementation of sage (*Salvia officinalis*), mint (*Mentha spicata*) and thyme (*Thymus vulgaris*) oils on growth performance, lipid peroxidation level (melondialdehyde, MDA) and liver antioxidant enzyme activities (superoxide dismutase, SOD; catalase, CAT; glucose-6-phosphate dehydrogenase, G6PD; glutathione reductase, GR; glutathione-S-transferase, GST and glutathione peroxidase, GPx) in rainbow trout (*Oncorhynchus mykiss*) juveniles. For this purpose, triplicate groups of rainbow trout were fed daily ad libitum with diets containing sage, mint and thyme oils at 500, 1,000 and 1,500 mg kg(-1) for 60 days. While weight gain percentage of fish fed the diets containing sage and thyme oils was significantly higher than the control group, that of fish fed mint oil was the lowest. Similarly, specific growth rate was

found to be the highest in all groups of the sage and thyme oil feeding and the lowest in the mint groups. Moreover, feed conversion ratio was significantly higher in the mint oil administered groups. Survival rate was also significantly reduced in the fish fed the diet containing mint oil. It was observed that SOD, G6PD and GPx activities were significantly increased in liver tissues of all the treated fish groups compared to that of control diet-fed group. However, CAT, GST and GR activities were significantly decreased in experimental diet-fed fish groups at the end of the experiment. On the other hand, a significant reduction was found in MDA levels in the fish fed the diets with sage and thyme oils compared to control and mint diets on the 30th and 60th days of experiment. Overall, dietary inclusion of sage and thyme oils is effective in enhancing rainbow trout growth, reduction in MDA and least changing antioxidant enzyme activities at a low level of 500 mg kg⁻¹ diet, and they can be used as important feed supplements for rainbow trout production.” As taken from Sönmez AY et al. 2015. Fish Physiol. Biochem. 41(1), 165-175. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/25431274>

10.3. Sediment toxicity

No data available to us at this time

10.4. Terrestrial toxicity

Record for oils, spearmint:

Spec. Name	Sci. Name	Resp. Type	Media Type	Exp. Site	Dose#	Endpoint	Effect	Signif.	Dose
Spec. Common Name		Exp. Dur. (Days)	Test Loc.	Chem. Anal.	Res. Sample Unit	BAF/ BCF	Effect Meas.	Sig. Level	Dose Stat. Meth.
CAS #/Chemical: 8008795 Oils, Spearmint									
Algae, Moss, Fungi									
Trichoderma harzianum			CUL	CM		LOEL	MOR	SIG	F (1.5-2.5) ul/ml
Fungus		1	LAB	U			MORT	<0.05	
Trichoderma harzianum			CUL	CM		LOEL	MOR	SIG	F (1.5-2.5) ul/ml
Fungus		1	LAB	U			MORT	<0.05	
Trichoderma harzianum			CUL	CM		LOEL	MOR	SIG	F (1.5-2.5) ul/ml
Fungus		1	LAB	U			MORT	<0.05	
Verticillium			CUL	CM		LOEL	MOR	SIG	F (1.5-2.5)

fungicola								ul/ml
Fungus	1	LAB	U			MORT	<0.05	
Trichoderma harzianum	MYC	CUL	CM		LOEL	POP	SIG	F (3.5-5.0) ul/ml
Fungus	21	LAB	U			ABND	<0.05	
Trichoderma harzianum	MYC	CUL	CM		LOEL	POP	SIG	F (3.5-5.0) ul/ml
Fungus	21	LAB	U			ABND	<0.05	
Trichoderma harzianum	MYC	CUL	CM		LOEL	POP	SIG	F (3.5-5.0) ul/ml
Fungus	21	LAB	U			ABND	<0.05	
Verticillium fungicola	MYC	CUL	CM		LOEL	POP	SIG	F (3.5-5.0) ul/ml
Fungus	21	LAB	U			ABND	<0.05	
Trichoderma harzianum	MYC	FLT	DA		LOEL	POP	SIG	F < 7 ul/eu
Fungus	21	LAB	U			ABND	<0.05	
Trichoderma harzianum	MYC	FLT	DA		LOEL	POP	SIG	F < 7 ul/eu
Fungus	21	LAB	U			ABND	<0.05	
Trichoderma harzianum	MYC	FLT	DA		LOEL	POP	SIG	F < 7 ul/eu
Fungus	21	LAB	U			ABND	<0.05	
Verticillium fungicola	MYC	FLT	DA		LOEL	POP	SIG	F < 7 ul/eu
Fungus	21	LAB	U			ABND	<0.05	
Trichoderma	MYC	CUL	CM		LOEL	POP	SIG	F (0.5-2.5)

harzianum								ul/ml
Fungus	1	LAB	U			ABND	<0.05	
Trichoderma harzianum	MYC	CUL	CM		LOEL	POP	SIG	F (0.5-2.5) ul/ml
Fungus	1	LAB	U			ABND	<0.05	
Trichoderma harzianum	MYC	CUL	CM		LOEL	POP	SIG	F (0.5-2.5) ul/ml
Fungus	1	LAB	U			ABND	<0.05	
Verticillium fungicola	MYC	CUL	CM		LOEL	POP	SIG	F (0.5-2.5) ul/ml
Fungus	1	LAB	U			ABND	<0.05	

As taken from the EPA ECOTOX database.

“The aim of this study was to evaluate the acaricidal activities of spearmint oil and carvone derivatives against house dust mites using contact and fumigant toxicity bioassays to replace benzyl benzoate as a synthetic acaricide. Based on the LD50 values, the contact toxicity bioassay revealed that dihydrocarvone (0.95 and 0.88 µg/cm²) was 7.7 and 6.8 times more toxic than benzyl benzoate (7.33 and 6.01 µg/cm²) against *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*, respectively, followed by carvone (3.78 and 3.23 µg/cm²), spearmint oil (5.16 and 4.64 µg/cm²), carveol (6.00 and 5.80 µg/cm²), and dihydrocarveol (8.23 and 7.10 µg/cm²). Results of the fumigant toxicity bioassay showed that dihydrocarvone (2.73 and 2.16 µg/cm²) was approximately 4.0 and 4.8 times more effective than benzyl benzoate (11.00 and 10.27 µg/cm²), followed by carvone (6.63 and 5.78 µg/cm²), carveol (7.58 and 7.24 µg/cm²), spearmint oil (9.55 and 8.10 µg/cm²), and dihydrocarveol (9.79 and 8.14 µg/cm²). Taken together, spearmint oil and carvone derivatives are a likely viable alternative to synthetic acaricides for managing house dust mites.” As taken from Yang JY et al. 2014. *Planta Med.* 80(2-3), 165-170. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24488719>

“The nematocidal activity and chemical characterization of aqueous extracts and essential oils of three mint species, namely, *Mentha × piperita*, *Mentha spicata*, and *Mentha pulegium*, were investigated. The phytochemical analysis of the essential oils was performed by means of GC-MS, whereas the aqueous extracts were analyzed by LC-MS. The most abundant terpenes were isomenthone, menthone, menthol, pulegone, and carvone, and the water extracts yielded mainly chlorogenic acid, salvianolic acid B, luteolin-7-O-rutinoside, and rosmarinic acid. The water extracts exhibited significant nematocidal activity against *Meloidogyne incognita*, and the EC50/72h values were calculated at 1005, 745, and 300 mg/L for *M. × piperita*, *M. pulegium*, and *M. spicata*, respectively. Only the essential oil from *M. spicata* showed a nematocidal activity with an EC50/72h of 358 mg/L. Interestingly, menthofuran and carvone showed EC50/48h values of 127 and 730 mg/L, respectively. On the other hand, salicylic acid, isolated in the aqueous extracts, exhibited EC50 values at 24 and 48 h of 298 ± 92 and 288 ± 79 mg/L, respectively.” As taken from Caboni P

et al. 2013. J. Agric. Food Chem. 61(41), 9784-8. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24050256>

"Cell cultures can be a potent and strong tool to evaluate the insecticidal efficiency of natural products. Plant essential oils have long been used as the fragrance or curative products around the world which means that they are safer to be used in close proximity of humans and mammals. In this study, a midgut cell line, developed from *Rhynchophorus ferrugineus* (RPW-1), was used for screening essential oils from nine different plants. Assays revealed that higher cell mortality was observed at 500 ppm which reached to 86, 65, 60, 59, 56, 54, 54, 53, and 53%, whereas lowest cell mortality at 1 ppm remained at 41, 23, 20, 17, 16, 15, 14, 13, and 10%, for *Azadirachta indica*, *Piper nigrum*, *Mentha spicata*, *Cammiphora myrrha*, *Elettaria cardamomum*, *Zingiber officinale*, *Curcuma longa*, *Schinus molle*, and *Rosmarinus officinalis*, respectively. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell proliferation assay revealed the percentage of cell growth inhibition was highest at 500 ppm and remained at 48, 45, 42, 37, 34, 29, 24, 22, and 18% against *A. indica*, *P. nigrum*, *M. spicata*, *C. myrrha*, *E. cardamomum*, *Z. officinale*, *C. longa*, *S. molle*, and *R. officinalis*, respectively. Lowest LC₅₀ value (7.98 ppm) was found for *A. indica*, whereas the highest LC₅₀ (483.11 ppm) was against *R. officinalis*. Thus, in this study, essential oils of *A. indica* exhibited the highest levels of toxicity, whereas those from *R. officinalis* exhibited the lowest levels of toxicity toward RPW-1 cells." As taken from Rizwan-UI-Haq M and Aljabr AM. 2015. In Vitro Cell Dev. Biol. Anim. 51(3), 281-286. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/25381034>

Honeybees - Contact acute 48 hour LD(50): >200 µg bee(-1)

Earthworms - Acute 14 day LC(50): > 1000 mg kg(-1)

As taken from BPDB, 2015.

"Natural compounds have been suggested as alternative sources for pediculosis capitis control. We aimed to investigate the chemical composition and evaluate the pediculicidal activity of spearmint, clove, cassia, thyme, eucalyptus, and anise essential oils in addition to sesame oil against human head lice in vitro. A filter paper contact bioassay method was used by applying 0.25 and 0.5 mg/cm(2) of each tested oil to filter paper in Petri dishes with 15 females head lice and another with ten nits. The lice mortalities were reported every 5 min for 180 min. The percentage of inhibition of hatch (PIH) was used to calculate ovicidal activity by daily microscopic inspections 5 days after the hatching of controls. Comparison with the widely used pediculicide (malathion) was performed. The most effective essential oil was spearmint followed by cassia and clove with KT50 values of 4.06, 7.62, and 12.12 at 0.5 mg/cm(2) and 8.84, 11.38, and 19.73 at 0.25 mg/cm(2), respectively. Thyme, eucalyptus, and anise were also effective adulticides with KT50 values of 18.61, 32.65, and 37.34 at 0.5 mg/cm(2) and 29.92, 43.16, and 45.37 at 0.25 mg/cm(2), respectively. Essential oils were also successful in inhibiting nymph emergence. Spearmint oil was the most effective, with a complete inhibition of emergence at 0.5 mg/cm(2). Sesame fixed oil did not show any adulticidal or ovicidal activity against head lice in vitro. The observed insecticidal activity was comparable to malathion. The results herein described the effectiveness of these essential oils as potential pediculicides for head lice control. Incorporation of essential oils in pediculicide formulations needs proper formulation and clinical trials." As taken from Yones DA et al. 2016. Parasitol. Res. 115(8), 3209-18. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/27112758>

"Essential oils from Greek *Mentha* species showed different chemical compositions for two populations of *M. pulegium*, characterized by piperitone and pulegone. *Mentha spicata* essential oil was characterized by endocyclic piperitenone epoxide, piperitone epoxide, and carvone. The bioactivities of these essential oils and their components have been tested against insect pests (*Leptinotarsa decemlineata*, *Spodoptera littoralis* and *Myzus persicae*), root-knot nematodes (*Meloydogine javanica*) and plants (*Lactuca sativa*, *Lolium perenne*, *Solanum lycopersicum*). The structure-activity relationships of these compounds have been studied including semi-synthetic endocyclic trans-carvone epoxide, exocyclic carvone epoxide, a new exocyclic piperitenone

epoxide and trans-pulegone epoxide. *Leptinotarsa decemlineata* feeding was affected by piperitenone and piperitone epoxide. *Spodoptera littoralis* was affected by piperitone epoxide and pulegone. The strongest nematocidal agent was piperitenone epoxide, followed by piperitone epoxide, piperitenone and carvone. Germination of *S. lycopersicum* and *L. perenne* was significantly affected by piperitenone epoxide. This compound and carvone epoxide inhibited *L. perenne* root and leaf growth. Piperitenone epoxide also inhibited the root growth of *S. lycopersicum*. The presence of a C(1) epoxide resulted in strong antifeedant, nematocidal and phytotoxic compounds regardless of the C(4) substituent. New natural crop protectants could be developed through appropriate structural modifications in the p-menthane skeleton." As taken from Kimbaris AC et al. 2017. Chem. Biodivers. 14(3). PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/27770481/>

"*Dermacentor reticulatus* ticks are among the most important arthropod vectors of zoonotic disease agents in Europe. Eleven essential oils, namely basil (*Ocimum basilicum*), bergamot (*Citrus bergamia*), clove bud (*Syzygium aromaticum*), citronella (*Cymbopogon winterianus*), creeping thyme (*Thymus serpyllum*), lavender (*Lavandula angustifolia*), lemon-scented gum (*Corymbia citriodora*), marjoram (*Origanum majorana*), peppermint (*Mentha piperita*), spearmint (*M. spicata*), and red thyme (*Thymus vulgaris*) were tested for repellency against adult *D. reticulatus* ticks at concentrations of 1 and 3%. Clove bud, creeping thyme and red thyme essential oils were the most efficient - repelling 83, 82 and 68% of ticks when diluted to 3%, respectively. The mixture of creeping thyme and citronella containing 1.5% of each showed higher repellency (91%) than individual essential oils at the concentration of 3%." As taken from Štefanidesová K et al. 2017. Ticks Tick Borne Dis. 8(5), 780-786. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/28645519>

10.5. All other relevant types of ecotoxicity

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that spearmint oils (CAS RN 8008-79-5) are of uncertain bioaccumulative potential in the environment.

Data accessed August 2017 on the OECD website: <http://webnet.oecd.org/CCRWeb/Search.aspx>
EPISuite provides the following data:

Bioaccumulation Estimates (BCFBAF v3.01):

Log BCF from regression-based method:	1.455 (BCF = 28.51 L/kg wet-wt)
Log Biotransformation Half-life (HL):	-0.2776 days (HL = 0.5277 days)
Log BCF Arnot-Gobas method (upper trophic):	1.637 (BCF = 43.35)
Log BAF Arnot-Gobas method (uppertrophic):	1.637 (BAF = 43.35)
log Kow used:	2.71 (expkow database)

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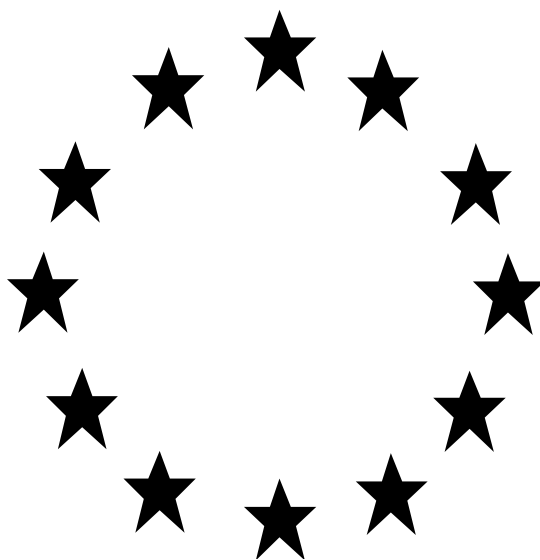
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12. Other information

No data available to us at this time.

13. Last audited

September 2022



Draft Assessment Report (DAR)

- public version -

**Initial risk assessment provided by the rapporteur Member State
Sweden for the existing active substance**

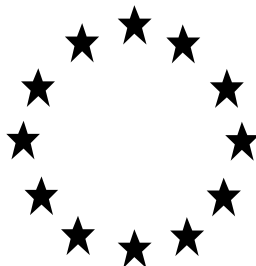
SPEARMINT OIL

**of the fourth stage of the review programme
referred to in Article 8(2) of Council Directive 91/414/EEC**

Volume 3, Annex B, part 2, B.6

August 2008

Draft Assessment Report



SPEARMINT OIL

Volume 3

Annex B.6

Toxicology and Metabolism

Rapporteur Member State: Sweden

June 2007

KEMI

Kemikalieinspektionen
Swedish Chemicals Agency

Volume 1

Level 1: Statement of subject matter and purpose for which the monograph was prepared

Level 2: Reasoned statement of the overall conclusions drawn by the Rapporteur Member State

Appendix 1: Standard terms and abbreviations

Appendix 2: Specific terms and abbreviations

Appendix 3: List of endpoints

Level 3: Proposed decision with respect to the application for inclusion of the active substance in Annex I

Level 4: Further information to permit a decision to be made, or to support a review of the conditions and restrictions associated with the proposed inclusion in Annex 1

Volume 2

Annex A: List of the tests and studies submitted and of information available

Volume 3

Annex B: RMS summary, evaluation and assessment of the data and information

Annex B.1: Identity

Annex B.2: Phys/chem.

Annex B.3: Data application and further information.

Annex B.4: Proposal for classification and labelling

Annex B.5: Analytical method

Annex B.6: Toxicology and metabolism

Annex B.7: Residues in crop

Annex B.8: Fate and behaviour

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Appendix 1: Standard terms and abbreviations

Appendix 2: Specific terms and abbreviations

Volume 4

Annex C: Confidential information and summary and assessment of information relating to the collective submission of dossiers

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WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

B.6 Toxicology and metabolism

The proposed use of Spearmint Oil, containing approximately 80 % w/w of l-carvone, is the anti-sprouting use on potatoes.

There is no specific formulation made, as the speciality contains only the essential oil of *Mentha spicata*. For that reason, this dossier is a mixed application dossier including all the available data on the active ingredient (normally included in Annex II) and the risk assessment of Spearmint Oil (normally included in Annex III).

In the safety evaluation of carvone prepared by WHO Food Additive Series 28, 42, and FAO Nutrition Meetings Report Series 44a, per capita intake of carvone was estimated from data derived from surveys in Europe (International Organization of the Flavour Industry, 1995) and the United States (US National Academy of Sciences, 1987). The estimated total daily per capita intake of carvone (No. 381, stereochemistry unspecified) and related substances (para-menthan-2-one, para-menthan-2-ol, dihydrocarvone, dihydrocarveol, dihydrocarvyl acetate, carveol, carvyl acetate and carvyl propionate) from use as flavouring agents is 2.8 mg/person in Europe and 10 mg/person in the United States. Carvone accounts for approximately 99% of the total annual per capita intake of this group of substances when used as flavouring agents in Europe and 96% in the United States.

l-Carvone or (-)-carvone (55-85%) has been reported in the oils of *Mentha* (spearmint). d-Carvone or (+)-carvone (20-75%) has been reported in *Carum* (caraway) and *Anethum* (dill) (Maarse et al., 1994). Quantitative data on the natural occurrence have been reported for five of these substances; dihydrocarvone, dihydrocarvyl acetate, carvone, carveol and carvyl acetate, which indicate that they are consumed predominantly in traditional foods (Stofberg & Kirschner, 1985; Stofberg & Grundschober, 1987 cited in WHO Food Additives Series No 42).

The notifier has performed acute studies on Spearmint Oil. Repeat dose studies have been performed with the primary component l-carvone (approximately 75% concentration in Spearmint Oil) and reported in the open literature, allowing assessment of both short-term and long-term risk. Additionally, these studies allow a toxicologically favourable comparison with d-carvone, on which more studies have been performed and reported in the public domain. This assessment report on Spearmint Oil and its primary component l-carvone, therefore also relies on read-across to public domain d-carvone data where appropriate. This is reasonable as there are no known indications that l-carvone is inherently more toxic than d-carvone after repeat exposure; indeed, the available data indicate that l-carvone is somewhat less toxic than d-carvone. As Spearmint Oil also contains a few percent of d-limonene, a presentation of the toxicological data on d-limonene has also been included in Appendix 1. d-Limonene is structurally very similar to carvone and read-across can be used in some cases, for example as an indicator of likely absorption, distribution and excretion.

A special document has been submitted from the European Commission "concerning the data requirements for active substances of plant protection products made from plants or plant extracts" Sanco/10472. As the active

substance is a plant extract some extra information are needed e.g. geographic origin, growth stage, growing conditions, microbiological quality of the harvest plants, detection and identification of possible contaminants such as heavy metals, toxins and pesticides. This information is given in Annex B.3.

B.6.1 Absorption, distribution, metabolism and excretion (toxicokinetics) (Annex IIA.5.1)

No ADME studies on Spearmint Oil has been conducted.

The notifier has submitted some studies from the public literature which gives some more information on carvone, sometimes the stereochemistry is known and sometimes it is unspecified, all the bibliographic data reported below are from the IPCS-INCHEM evaluations (WHO Food Additive Series 28, 42, and FAO Nutrition Meetings Report Series 44a).

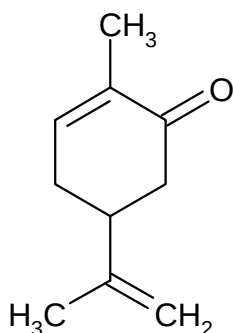


Fig. B.6.1-1. Structural formula of l-carvone, main component of Spearmint oil.

A racemic mixture of carvone was reported to undergo side-chain oxidation in rabbits. Each of six male rabbits was given approximately 2 g (d and l)-carvone orally in water. Urine collected over three days was separated into neutral, acidic, and phenolic fractions. After hydrolysis of the glucuronic acid and sulfate conjugates, chromatographic analysis of the neutral fraction revealed the presence of (+)-9-hydroxycarvone (10%) (Williams, 1959; Ishida et al., 1989).

Oxidation of the side-chain has been observed for other acyclic (Nishizawa et al., 1987) and alicyclic ketones (Williams, 1940; Nelson et al., 1992), with cytochrome P450 acting as a catalyst in vitro (Madyastha & Raj, 1990); however, no effects were observed on the activity of either cytochrome P450 or cytochrome b5 in male albino rats given (-)-carvone at 600 mg/kg bw per day orally for three days (Moorthy et al., 1989).

In a study to examine the ability of carvone and carvyl derivatives to induce enzymes for the detoxification of carcinogens (Wattenberg et al., 1989; Lam & Zheng, 1991, 1992), glutathione S-transferase activity increased and glutathione content decreased in various tissues of groups of A/J mice given repeated oral doses of carvone and 10 carvyl derivatives. Groups of four female A/J mice were given three oral doses, each containing 20 mg of a carvyl derivative in cottonseed oil, by gavage over two days. The test substances included alpha,beta-unsaturated ketones, (+)-carvone, 8,9-dihydro-carvone, 9-hydroxycarvone, 9-acetoxycarvone, the unconjugated ketones 2,3-dihydrocarvone and carvomenthone, and five carvyl alcohol derivatives. Cytosolic glutathione S-transferase

activity and acid-soluble sulphhydryl levels were measured in mouse liver, forestomach, lung, and small and large bowel mucosa. Treatment with carvone and, to a lesser degree, other α,β -unsaturated ketones increased the activity of glutathione S-transferase in all tissues by two to four times over that in controls and in animals treated with other carvyl derivatives. Carvone intake was associated with a decrease in glutathione content in the liver, lung, and large-bowel mucosa (Zheng et al., 1992). Carvone rapidly conjugated with glutathione in the absence of glutathione S-transferase (Portoghesi et al., 1989).

Carvone is also metabolized in rabbits by hydrogenation of the endocyclic double bond to yield 8-menthen-2-ol (dihydrocarveol), which is excreted unchanged (Fischer & Bielig, 1940).

Carvone has been detected unchanged in the urine of humans, presumably arising from its dietary intake (Zlatkis et al., 1973).

As noted previously, d-limonene is structurally similar to carvone, and would be expected to be absorbed, distributed and excreted in a similar way. The notifier has submitted public domain information on d-limonene which is fully summarised in Appendix 1. All the bibliographic data reported below become from the IPCS-INCHEM evaluation (WHO Food Additive Series 30).

Summary and conclusions on absorption, distribution, metabolism and excretion studies in mammals.

It is expected that l-carvone would be absorbed, distributed and excreted in a similar way to structurally similar d-limonene. After oral administration in rats, the latter is rapidly absorbed and distributed in blood serum, liver and kidneys, along with its metabolites (1-2 h), followed by excretion (48 h) in the urine (60%), faeces (5%) and expired air (2%). After topical administration in rats, partial absorption (48% of the radioactivity was recovered in the skin) and subsequent distribution in the gastro-intestinal tract (0.1-0.4% dose/g), liver and kidneys (0.08-0.2% dose/g), and thyroid and fat (0.02-0.06% dose/g), is also rapid (3-6 h), followed by excretion (24-72 h) in the urine (8-12%), faeces (1-3%) and expired air (14-18%).

Although the d-limonene absorption, distribution and excretion data are indicative for l-carvone, they provide no direct information on the percentage absorption of l-carvone after oral, dermal or inhalation exposure, the distribution of l-carvone or the rate and routes of elimination from the body. Therefore, default values of 50, 100 and 100% for oral, dermal and inhalation absorption will be used for the exposure and risk assessment calculations for operators, workers and bystanders.

The terpenoid alcohols resulting from ester hydrolysis and their corresponding ketones are metabolized like other alicyclic terpenoid ketones and secondary alcohols. Five detoxification pathways have been identified:

- reduction of the ketone followed by conjugation of the resulting alcohol with glucuronic acid;
- side-chain oxidation, yielding polar metabolites which may be conjugated and excreted;
- glutathione conjugation of ketones, followed by excretion;
- hydrogenation of the endocyclic double bond of carveol; and
- excretion of unchanged carvone.

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Agres REP: Toxicology and Metabolism

B.6.2 Acute toxicity including irritancy and skin sensitization (Annex IIA 5.2)**B.6.2.1 Oral****B.6.2.1.1 Rat**

REFERENCE 01:	T Mullaney (2005) HUILE DE MENTHE HN: ACUTE ORAL TOXICITY IN THE RAT – ACUTE TOXIC CLASS METHOD PROJECT NUMBER: 1620/043
Test substance/purity:	Spearmint Oil / 74% L-Carvone
Guideline/GLP:	OECD No 423/Yes
Deviation:	
Acceptability:	Yes
Species/strain:	Sprague-Dawley CD rats
Doses/no. of animals:	Single dose: 2000 mg kg ⁻¹ bw; 3+3 females
Administration way/vehicle:	Orally by gavage
Test system:	The animals were observed for deaths or overt signs of toxicity 0.5, 1, 2 and 4 hours after dosing and subsequently once for fourteen days. Individual bodyweights were recorded prior to dosing and seven and fourteen days after treatment. All animals were subjected for gross pathological examination.

Findings:

There were no deaths. Signs of systemic toxicity noted during the study were hunched posture, lethargy, ataxia, piloerection and red/brown staining around the snout. All animals appeared normal two days after dosing. No abnormal abnormalities were noted at necropsy.

Conclusion:

The acute oral median lethal dose (LD₅₀) of Spearmint Oil was estimated to be greater than 2000 mg kg⁻¹ bw. The ataxia, lack of coordination, indicates that at this high limit test dose level part of the nervous system was affected temporarily on the day of exposure and the day after.

The test material does not meet the criteria for classification according to EU labelling.

B.6.2.2 Percutaneous**B.6.2.2.1 Acute dermal toxicity in rat**

REFERENCE 01:	T Mullaney (2005) HUILE DE MENTHE HN: ACUTE DERMAL TOXICITY (LIMIT TEST) IN THE RAT PROJECT NUMBER: 1620/044
Test substance/purity:	Spearmint Oil / 74% L-Carvone
Guideline/GLP:	OECD Guideline No. 402/Yes
Deviation:	

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Acceptability:	Yes
Species/strain:	Sprague-Dawley CD rats
Doses/no. of animals:	2000 mg kg ⁻¹ bw; 5 males and 5 females
Administration way/vehicle:	Single, semi-occluded dermal application to intact skin
Exposure time:	24 hours
Test system:	The animals were observed for deaths or overt signs of toxicity 0.5, 1, 2 and 4 hours after dosing and subsequently once daily. Clinical signs and body weight development were monitored during the study. All animals were subjected to gross necropsy.

Findings:

There were no deaths, signs of systemic toxicity or signs of dermal irritation. All animals showed expected gains in bodyweight over the study period. No abnormalities were noted at necropsy.

Conclusions:

The acute dermal median lethal dose (LD₅₀) of Spearmint Oil was estimated to be greater than 2000 mg kg⁻¹ bw. The test material does not meet the criteria for classification according to EU labelling.

B.6.2.3 Inhalation**B.6.2.3.1 Inhalation in rat**

REFERENCE 01:	D R Griffiths and P Watson (2006) HUILE DE MENTHE HN: ACUTE INHALATION TOXICITY (NOSE ONLY) STUDY IN THE RAT PROJECT NUMBER: 1620/045
Test substance/purity:	Spearmint Oil / 74% L-Carvone
Guideline/GLP:	OECD Guideline No. 403/Yes
Deviation:	
Acceptability:	Yes
Species/strain:	Sprague-Dawley CrI:CD® (SD) IGS BR
Doses/no. of animals:	5.43 mg/L air; 5 males and 5 females
Administration way:	Inhalation, nose only
Exposure time:	4 hours
Test system:	The rats were nose only exposed to an aerosol for four hours, followed by a fourteen day observation period. The exposure chamber concentration of the test material was measured off-line by gas chromatography. Mean achieved concentration was 5.43±0.20 mg/mL and the nominal concentration 18.7 mg/mL. Mean Mass Median Aerodynamic Diameter was 3.32 µm, inhalable fraction <4 µm was 61.3 % and geometric standard deviation 1.92. Animals were observed for clinical signs at hourly intervals during exposure, immediately on removal from restraining tubes at the end of exposure, one hour after termination of exposure and subsequently once daily for fourteen days.

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Findings:

There were no deaths.

Common abnormalities noted during the study included increased respiratory rate, hunched posture, pilo-erection and wet fur. There were frequent instances of ataxia, occasional instances of noisy respiration and red/brown staining around the snout and isolated occurrence of laboured respiration and fur loss around the head and snout. Animals recovered quickly such that no significant observations were apparent from Days 1 to 5 post-exposure. Normal bodyweight development was noted during the study. Apart from an isolated instance of dark patches on the lungs, no abnormalities were noted at necropsy.

Conclusion:

No death occurred in a group of ten rats exposed to a mean achieved atmosphere concentration of 5.43 mg/L for four hours. It was therefore considered that the acute inhalation median lethal concentration (4 h LC₅₀) of Spearmint Oil was greater than 5.43 mg/L. The ataxia, lack of coordination, indicates that at this high limit test dose level part of the nervous system was affected temporarily on the day of exposure. The test material, Spearmint Oil, does not meet the criteria for classification according to EU labelling and will not require labelling for inhalation toxicity.

B.6.2.4 Skin irritation**B.6.2.4.1 Rabbit**

REFERENCE 01:	T Mullaney (2005) HUILE DE MENTHE HN: ACUTE DERMAL IRRITATION IN THE RABBIT
Test substance/purity:	PROJECT NUMBER: 1620/046
Guideline/GLP:	Spearmint Oil / 74% L-Carvone
Deviation:	OECD guideline No. 404
Acceptability:	Yes
Species/strain:	Albino rabbits
Doses/no. of animals:	3 rabbits, sex not stated
Administration way/vehicle:	Dermal/90% v/v aqueous preparation
Exposure time:	4 hours
Test system:	0.5 ml of the test material was introduced under 2.5 cm x 2.5 cm cotton gauze patch and placed in position on the horn skin. One hour following the removal of the patches, and 24, 48, and 72 hours later, the test site was examined for evidence of primary irritation.

Findings:

Spearmint Oil produced no evidence of skin irritation.

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Table B.6.2.5.1-1: Individual and Total Scores

Conclusion:

Spearmint Oil produced a primary irritation index of 0.0 and was classified as non-irritant to rabbit skin according to the Draize classification scheme. No corrosive effects were noted.

The test material, Spearmint Oil, does not meet the criteria for classification as irritant or corrosive according to EU labelling regulations.

B.6.2.5 Eye irritation**B.6.2.5.1 Rabbit**

REFERENCE 01:	T Mullaney HUILE DE MENTHE HN: ACUTE EYE IRRITATION IN THE RABBIT PROJECT NUMBER: 1620/047
Test substance/purity:	Spearmint Oil / 74% L-Carvone
Guideline/GLP:	OECD Guideline 405/Yes
Deviation:	
Acceptability:	Yes
Species/strain:	New Zealand White rabbits
Doses/no. of animals:	0.1 ml in one eye; 1+2 males
Administration way/vehicle:	Test material was placed into the conjunctival sac of the right eye/ No vehicle
Exposure time:	
Test system:	Initially, a single rabbit was treated. Two additional rabbits were treated after administration of one drop of local anaesthetic. Assessment of ocular damage/irritation was made 1, 24, 48 and 72 hours following treatment.

Findings:

A single application of the test material to the non-irrigated eye of three rabbits produced iridial inflammation and moderate conjunctival irritation. Two treated eyes appeared normal at the 48-hour observation and the remaining treated eye appeared normal at the 72-hour observation. See Table B.6.2.5.1-1 & 2.

Table B.6.2.5.1-1: Individual Scores and Individual Total Scores for Ocular Irritation

Rabbit No and sex	92 Male				8 Male				9 Male			
	Initial pain reaction=3				Initial pain reaction =0 +				Initial pain reaction =0 +			
Time after treatment	1 Hour	24 Hours	48 Hours	72 Hours	1 Hour	24 Hours	48 Hours	72 Hours	1 Hour	24 Hours	48 Hours	72 Hours
CORNEA												
E=Degree of Opacity	0	0	0	0	0	0	0	0	0	0	0	0
F=Area of Cornea Involved	0	0	0	0	0	0	0	0	0	0	0	0
Score (ExF) x 5	0	0	0	0	0	0	0	0	0	0	0	0
IRIS												
D	0	0	0	0	1	0	0	0	0	0	0	0
Score (D x 5)	0	0	0	0	5	0	0	0	0	0	0	0
CONJUNCTIVAE												
A=Redness	1	1	0	0	2	1	0	0	2	1	1	0
B=Chemosis	1	0	0	0	1	0	0	0	1	0	0	0
C=Discharge	1	0	0	0	2	1	0	0	2	1	0	0
Score (A + B + C) x 2	6	2	0	0	10	4	0	0	10	4	2	0
Total Score	6	2	0	0	15	4	0	0	10	4	2	0

+ = One drop of local anaesthetic instilled into both eyes 1 to 2 minutes before treatment

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Table B.6.2.5.1-2: Individual Total Scores and Group Mean Scores for Ocular Irritation

Rabbit Number and sex	Individual total scores at:			
	1 Hour	24 Hours	48 Hours	72 Hours
92 Male	6	2	0	0
8 Male	15	4	0	0
9 Male	10	4	2	0
Group Total	31	10	2	0
Group Mean Score	10.3	3.3	0.7	0.0

Conclusion:

Spearmint Oil produced a maximum group mean score of 10.3 and was classified as mild irritant (Class 4 on 1 to 8 scale) to the rabbit eye according to a modified Kay and Calandra classification systems.

The test material did not meet the criteria for classification as irritant according to EU labelling regulation.

B.6.2.6 Skin sensitization**B.6.2.6.1 Skin sensitization test in guinea pigs (Local Lymph Node Assay)**

REFERENCE 01:	T Mullaney HUILE DE MENTHE HN: LOCAL LYMPH NODE ASSAY IN THE MOUSE PROJECT NUMBER: 1620/048
Test substance/purity:	Spearmint Oil / 74% L-Cardene
Guideline/GLP:	OECD Guideline 4029/Yes
Deviation:	
Acceptability:	Yes
Species/strain:	Mouse/CBA/Ca
Doses/no. of animals:	0%, 25%, 50% and 100% v/v in acetone/olive oil 4:1/ 4 females per group
Administration way/vehicle:	Dermal application to the dorsal surface of the ear
Exposure time:	Follow the guideline
Test system:	The mice were treated by daily application of 25µl of test material for three consecutive days (Days 1, 2, 3). A further group of four mice received the vehicle alone in the same manner. Five days following the first topical application of the test material (Day 6) all mice were injected via the tail vein with 250 µl phosphate buffered saline containing 3 H-methyl thymidine giving a total of 20 µl Ci to each mouse. All animals were observed twice daily on Day1, 2, and3 and on a daily basis on Days 4, 5, and 5. Any signs of toxicity or signs of ill health during the test were recorded. The body weight of each mouse was recorded on Day 1 (prior to dosing) and Day 6 (prior to termination).

Findings:

No death or signs of systemic toxicity were noted and body weight changes for test animals were similar to those observed in control animals.

The Stimulation Index (SI) expressed as the radioactive incorporation for each treatment group divided by the mean radioactive incorporation of the vehicle control group are as follows:

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Annex B.6: Toxicology and Metabolism

Table B.6.2.6.1-1 Results of Skin sensitization test

Concentration (% v/v) in Acetone/olive oil 4:1	Stimulation Index (SI)	Result
25	3.08	Positive
50	6.43	Positive
100	7.82	Positive

Conclusion:

Spearmint Oil was considered to be a sensitizer under the conditions of the test.

The test material was classified as a sensitizer according to EU labelling regulations. The symbol “Xi”, indication of danger irritant and the risk phrase R 43 “May cause sensitisation by skin contact” are therefore required.

B.6.2.7 Summary and conclusions on acute toxicity studies

The results of the acute toxicity, irritation and sensitisation studies are presented in Table 6.2.7-1

Table B.6.2.7-1: Overview of acute toxicity studies with HUILE DE MENTHE (Spearmint Oil)

Parameter	Species	Result	Reference
Oral LD ₅₀	Rat	>2 000 mg kg ⁻¹	Mullaney, 2005
Dermal LD ₅₀	Rat	>2 000 mg kg ⁻¹	Mullaney, 2005
Inhalation 4 hr LC ₅₀	Rat	>5.43 mg/L	Griffiths and Watson, 2006
Skin irritation	Rabbit	Non-irritant	Mullaney, 2005
Eye irritation	Rabbit	Mild irritant	Mullaney, 2005
Skin sensitisation	Mouse	Sensitizer	Mullaney, 2005

The acute oral, dermal, and inhalation toxicity tests were only performed in one species, rat. The ataxia, seen in the oral and the inhalation study, indicates that part of the nervous system was affected. This finding is supported by a study by Gordon *et al.*, 1982 (in the safety evaluation of Carvone prepared by WHO (Food Additive Series No. 28) where (+)-Carvone (D-Carvone) was administered by intraperitoneal injection to mice in graded doses (not given) up to 800 mg/kg body weight at which high dose level significant convulsive effects occurred.

On the basis of the above results, HUILE DE MENTHE/Spearmint Oil does not need to be classified with respect to its acute oral, dermal and inhalation toxicity. Nevertheless, as HUILE DE MENTHE/Spearmint Oil is composed of alicyclic hydrocarbons, has a kinematic viscosity of 2.95×10^{-6} m²/sec at 20°C and has a surface tension of 31.13 mN/m, it does present a theoretical aspiration hazard to humans; on this basis it meets the criteria for assignment of the symbol “Xn”, indication of danger harmful and the risk phrase R 65 Harmful: may cause lung damage if swallowed. HUILE DE MENTHE/Spearmint Oil did not meet the criteria for classification as irritant to skin or eyes according to EU labelling regulation. HUILE DE MENTHE/Spearmint Oil was a skin sensitizer in the animals tested and the test material was classified as a sensitizer according to EU labelling regulations. The symbol “Xi”, indication of danger irritant and the risk phrase R 43 “May cause sensitisation by skin contact” are therefore required.

Complementary information of acute toxicity of carvone cited in WHO Food Additive Series 28, 42, and FAO Nutrition Meetings Report Series 44a

Table 5.2.1.2: Summary of acute toxicity studies published on carvone

Species	Route	LD ₅₀	Purity	reference
Mouse	S.C.	2675 mg/kg bw	NS	Wenzel & Ross, 1957
Rat	Oral	1640 mg/kg bw ^a	NS	Jenner et al., 1964
Rat	Oral	3710 mg/kg bw ^b	NS	Opdyke, 1978
Guinea-pig	Oral	766 mg/kg bw ^c	NS	Jenner et al, 1964
Rabbit	Dermal	3860 mg/kg bw	NS	Opdyke, 1978

S.C. = Subcutaneous. NS = Not stated. ^a Oral LD50 reported on "carvone", identified as *p*-mentha-6,8-dien-2-one. ^b Oral LD50 reported on *d*-carvone. ^c Dermal LD50 reported as 4 ml/kg body weight for *d*-carvone.

Single intraperitoneal injections of *d*-carvone to male Swiss- Webster albino mice did not result in any observed hepatotoxic or pulmonary effects (Gordon et al. 1982).

B.6.3 Short-term toxicity (Annex IIA 5.3)

No short term toxicity study results are available in the literature, however, as the main component of Spearmint Oil is *l*-carvone and for a minor part limonene, this part includes the bibliographic data on both these components, as representative of the toxicity of the essential oil. (Limonene data see Appendix I)

For carvone, all the bibliographic data reported below comes from the IPCS-INCHEM evaluations (WHO Food Additive Series 28, 42 and 44a. Sometimes the isomeric chemistry is specified and sometimes not. The RMS has estimated NOAELs from the results that are available in these documents. Note that the NOAELs are not based on the original studies!

B.6.3.1 Oral

No original studies submitted. The studies referred to below are from the open literature.

B.6.3.1.1 Oral studies in rat

Study 1) Groups of five male and five female Osborne-Mendel rats were maintained on diets containing carvone (*p*-mentha-6,8-dien-2-one) (isomer not specified) at a level of 1000 ppm (approximately equivalent to 50 mg/kg bw/day) for 27-28 weeks (Hagan et al., 1967, and

Study 2) at a level of 2500 ppm (approximately equivalent to 125 mg/kg bw/day) for one year. No adverse effects were observed in these rats as judged by growth, appearance, food intake, haematology, final body weights, or gross and microscopic examination of the major organs (Hagan et al., 1967.

Study 3) Rats maintained on a diet containing carvone at a level of 10,000 ppm (approximately equivalent to 500 mg/kg bw/day) for 16 weeks showed growth depression and testicular atrophy (Hagan et al., 1967).

Study 4) Groups of five F344/N rats (6-7 weeks old) of each sex were administered 0, 150, 328, 723, 1,590 or 3,500 mg/kg body weight d-carvone in corn oil by gavage, 5 days per week, for 12 doses over 16 days. The animals were housed five per cage and water and feed were given ad libitum. The rats were observed twice per day and were weighed on days 1, 7 and 14 and at the end of the study. A necropsy was performed on all animals. All rats that received 1,590 or 3,500 mg/kg body weight died during the first week of the study. The male rats that received 723 mg/kg body weight had 9% lower mean necropsy bodyweight than controls. Relative liver weights for male rats in the group receiving 723 mg/kg body weight were significantly higher than those of the vehicle controls. Relative kidney weights for rats of either sex that received 328 or 723 mg/kg body weight were significantly higher than those for the vehicle controls. The relative weights of the thymus of male rats at 723 mg/kg body weight and of brain in female rats at 723 mg/kg body weight were decreased. No histopathology was reported (NTP, 1989).

Study 5) Groups of 10 female and 30 male F 344/N rats (4-5 weeks old) were administered 0, 93, 375, or 1,500 mg/kg body weight d-carvone in corn oil by gavage, 5 days per week, for 13 weeks. Additional groups of 10 rats of each sex were included at dose levels of 187 or 750 mg/kg body weight on the same schedule. Animals were housed five per cage and allowed feed and water ad libitum. The rats were observed twice per day and were weighed at the beginning of the study, once per week thereafter, and at the end of the study. A necropsy was performed on all animals. All rats that received 1,500 mg/kg body weight died during the first weeks and 9/10 males and 10/10 females that received 750 mg/kg body weight died before the termination of the study (weeks 7-12). Final mean body weights of male rats that received 187 or 375 mg/kg body weight were lower (7% or 11%, respectively) than those of the controls. The relative liver weights for dosed males and females that received 93, 187 or 375 mg/kg body weight were increased. The relative kidney weights of males administered 187 or 375 mg/kg, and of females that received 375 mg/kg body weight, were also increased. The relative weight of the right testis and lungs/bronchi of males were increased at dose levels of 187 or 375 mg/kg body weight. In females receiving 375 mg/kg body weight the relative brain weights were decreased. Histopathology was performed on vehicle controls, rats receiving 375 mg/kg body weight and all animals dying or killed before the end of the study. Testicular degeneration was seen in the male rats at 750 mg/kg body weight. This lesion was characterized by moderate to marked loss of germinal epithelium in the seminiferous tubules and an absence of sperm in the epididymal ducts. In some rats, syncytial giant cells of spermatids were present in seminiferous tubules. Atrophy of the thymic cortex was present in rats of each sex which received 750 mg/kg body weight. Hyaline droplets and granular casts commonly associated with the accumulation of alpha₂μ-globulin were not observed in the kidney tissue of dosed rats. No further information was given on pathology (NTP, 1989).

Study 6) Groups of ten 6-week old male Wistar rats were administered d-carvone or l-carvone at levels of 80 or 160 mg/kg bw/day for 28 days. Plasma creatinine was determined in all rats on days 0, 13 and 28 using a specific HPLC-method. A dose related decrease in plasma creatinine was observed on day 28 in the rats receiving d-carvone. l-Carvone had no effect (Lam, 1988).

6.3.1.2 Oral toxicity studies in the mouse

Study 1) Groups of five B6CBF1 mice (7-8 weeks old) of each sex were administered 0, 150, 328, 723, 1,590 or 3,500 mg/kg body weight d-carvone in corn oil by gavage, 5 days per week, total 12 doses over 16 days. Animals were housed five per cage. Water and feed were given ad libitum. The mice were observed twice per day and were weighed before the study and on days 1, 7 and 14 and at the end of the study. A necropsy was performed on all animals. All mice receiving 1,590 or 3,500 mg/kg body weight died on day 2 or 3. At necropsy the mean body weights of the treated animals were similar to controls. Ataxia, impaired grasping reflex, ocular discharge, corneal opacity, body tremors, prostration, gasping, clonic or tonic convulsions, or impaired righting reflex were observed at 1,590 and 3,500 mg/kg body weight; and lacrimation, piloerection, hypoactivity, bradypnea, or ptosis were observed at 723, 1,590 and 3,500 mg/kg body weight. Relative liver weights were increased in all groups of dosed male mice and relative thymus weights were decreased in all groups of dosed female mice. Relative weights of brain, heart, right kidney, lungs and bronchi were not changed. Histological examinations were carried out on all animals in the control group and the highest dosed group. However, the lungs and livers of all dosed mice were examined. No compound-related changes were seen (details on pathology not given) (NTP, 1989).

Study 2) Groups of 10 female and 30 male B6CBF1 mice (5-7 weeks old) were given 0, 93, 375 or 1,500 mg/kg body weight d-carvone in corn oil by gavage, 5 days per week, for 13 weeks. Additional groups of 10 mice of each sex were administered 187 or 750 mg/kg body weight on the same schedule. Animals were housed five per cage and feed and water were available ad libitum. The mice were observed twice per day. The animals were weighed at the beginning of the study and then once per week, and at the end of the study. A necropsy was performed on all animals. All male and 8/10 female mice that received 1,500 mg/kg body weight and 1/10 males at 93 mg/kg body weight died during the first week of the study. An additional female mouse died during week 12 of the study. Final mean body weights of dosed and control animals were similar. Hypoactivity, ataxia, and hypersensitivity to touch were seen at 1,500 mg/kg body weight. The female that received 1,500 mg/kg body weight and survived to the end of the study had body tremors, hypersensitivity to touch, and impaired hind limb function during the study. The relative liver weights for male and female mice that received 750 mg/kg body weight were significantly greater than of vehicle controls. Relative weights of brain, thymus, lungs/bronchi, heart, right kidney, and right testis (males) were not altered. Histological examinations were carried out on vehicle controls, mice receiving 750 mg/kg body weight, and all animals that died or were killed before the end of the study. No compound-related changes were seen (details on pathology not given) (NTP, 1989).

B.6.3.2 Inhalation

No studies are available.

B.6.3.3 Dermal

No studies are available

B.6.3.4 Summary and conclusions on oral short-term toxicity studies on carvone

Note that no studies have been performed with Spearmint Oil or pure l-carvone and that RMS only has had access to studies from the open literature! This makes the estimated NOAELs rather rough.

Table B.6.3.4 – 1: Summaries of studies from the open literature.

Animals	Isomer	Dose level	Duration time	NOEL/NOAEL*	Organ effects	Ref
<i>Oral administration in rat</i>						
5 male + 5 female	Not specified	50 mg/kg bw/ day	27-28 weeks	NOAEL>50 mg/kg bw/day	No adverse effects judged by growth, appearance, food intake, haematology, final body weights, or gross and microscopic examination of the major organs	Hagan et al., 1967
5 male + 5 female	Not specified	125 mg/kg bw/ day	1 year	NOAEL>125 mg/kg bw/day	No adverse effects judged by growth, appearance, food intake, haematology, final body weights, or gross and microscopic examination of the major organs	Hagan et al., 1967
5 male + 5 female	Not specified	500 mg/kg bw/ day	16 weeks	NOAEL<500 mg/kg bw/day	Growth depression and testicular atrophy	Hagan et al., 1967
5 male + 5 female	d-carvone	0, 150, 328, 723, 1590, 3500 mg/kg bw/ day	5 days per week, for 12 doses over 16 days	NOAEL 150 mg/kg bw/day	Increase in kidney weight at 328 mg/kg bw/day followed by decrease in thymus and brain weight at 723 mg/kg bw/day. All rats receiving 1590 and 3500 mg/kg bw/day died.	NTP, 1989
40 males + 20 females	d-carvone	0, 93, 187, 375, 750, 1500 mg/kg bw/day	5 days per week, for 13 weeks	NOAEL<93 mg/kg bw/day	Decreased body weight, increased relative kidney and liver weight at 93 mg/kg bw/day, testis and lungs weights in male at 187 mg/kg bw/day, testinal degradation at 750 mg/kg bw/day. All rats receiving 1500 mg/kg bw/day died.	NTP, 1989

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Animals	Isomer	Dose level	Duration time	NOEL/NOAEL*	Organ effects	Ref
<i>Oral administration in mouse</i>						
5 animals/ group and sex	d-carvone	0, 150, 328, 723, 1590, 3500 mg/kg bw/ day	5 days per week, for 12 doses over 16 days	NOAEL < 150 mg/kg bw/day	Increased liver weight in male and decreased thymus weight in female at 150 mg/kg bw/day	NTP,1989
40 males + 20 females	d-carvone	0, 93, 187, 375, 750, 1500 mg/kg bw/ day	5 days per week, for 13 weeks	NOAEL 375 mg/kg bw/day	Increase in liver weight at 750 mg/kg bw/day. All rats receiving 1500 mg/kg bw/day died.	NTP,1989

* Note that this is an attempt to roughly estimate NOAELs as RMS only has had access to summaries of studies from the open literature!

Oral short-term toxicity

The oral short-term (and long term) toxicity for carvone has been studied in rat and mouse. The major detected adverse effects were growth depression, testicular atrophy, and increase in liver and kidney weight and decreased thymus weight. No reliable NOAEL can be established but taken the available studies together, the NOAEL seems to be in the range of 50 to 100 mg/kg bw /day based on decreased body weight and increased relative liver and kidney weight.

Inhalation short-term toxicity

There are no studies available with carvone.

Dermal short-term toxicity

There are no studies available with carvone.

B.6.4 Genotoxicity (Annex IIA 5.4)

No genotoxicity data on Spearmint Oil are available in the literature. However, as the main component of Spearmint Oil is l-carvone, carvone data can be regarded as representative of the essential oil.

For carvone, all the bibliographic data reported below are derived from the IPCS-INCHEM evaluations (WHO Food Additive Series 28(1991), 42(1999) and FAO Nutrition Meetings Report Series 44a(1967).

B.6.4.1 In vitro studies

All these studies were summarized by WHO [d-carvone and l-carvone-Food additives Series 28] as follows:

B.6.4.1.1 Bacterial assay for gene mutation study

Findings:

d-Carvone at concentrations up to 333 µg/plate was negative for induction of gene mutations in *Salmonella typhimurium* strains TA 100, TA 1535, TA 1537 and TA 98 when tested in a preincubation assay in the presence or absence of Aroclor 1254 induced male Sprague Dawley rat or Syrian hamster liver S9 (Mortelmans et al., 1986; NTP, 1989).

Conclusion:

It was concluded that d-carvone did not show any evidence of mutagenic activity in this bacterial test system at the dose levels indicated.

B.6.4.1.2 Test for gene mutation in mammalian cells

Findings:

d-Carvone induced slight increases in sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary (CHO) cells with and without addition of Aroclor 1254 induced male Sprague Dawley rat liver S9. Results were statistically positive but in two of three sister chromatid exchange trials there were no correlation of dose with response. This phenomenon of no dose/response relations also occurred in the second trial of the chromosomal aberration test conducted without S9. In the chromosomal aberration test without S9 a chemical-induced delay in cell cycle was observed.

This was not observed in the test for sister chromatid exchanges (NTP, 1989).

Conclusion:

The d-carvone results are not conclusive.

B.6.4.2 In vivo study

No available information

B.6.4.3 Summary and conclusions on genotoxicity testing

From one study it was concluded that d-carvone did not have any mutagenic activity in the bacteria test system.

The results from the in vivo studies with d-carvone looking for sister chromatid exchange and chromosomal aberrations were equivocal and no conclusions could be drawn.

There are no available data on l-carvone (see the footnote under the table!) or Spearmint Oil.

Table B.6.4.3 – 1: Results of assays for the genotoxicity of carvone

Substance	No.	End-point	Test object	Dose	Result	Reference
carvone*	381	Gene mutation	<i>S. typhimurium</i> TA1535, TA1537, TA98, TA100	3 µmol/plate	Negative	Florin et al. (1980)
l-carvone*	381	Gene mutation (preincubation)	<i>S. typhimurium</i> TA1535, TA98, TA100, TA1537	333 µg/plate	Negative	Mortelmans et al. (1986)

SPEARMINT OIL
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Substance	No.	End-point	Test object	Dose	Result	Reference
l-carvone*	381	Gene mutation	S. typhimurium TA1535, TA98, TA100, TA1537	333 µg/plate	Negative	National Toxicology Program (1990)
carvone*	381	Rec assay	Bacillus subtilis H17 (rec+) and M45 (rec-)	0.6 ml/disc	Negative	Matsui et al. (1989)
l-carvone*	381	Sister chromatid exchange	Chinese hamster ovary cells	502 µg/ml	Equivocal	National Toxicology Program (1990)
l-carvone*	381	Chromosomal aberration	Chinese hamster ovary cells	400 µg/ml	Equivocal	National Toxicology Program (1990)

* As far as RMS can find out all studies in No 381 are performed with d-carvone and not with l-carvone as the notifier has indicated in the table.

B.6.5 Long-term toxicity and carcinogenicity (Annex IIA 5.5)

No long-term toxicity and carcinogenicity data on Spearmint Oil are available in the literature, however, as the main component of Spearmint Oil is l-carvone, carvone data can be regarded as representative of the essential oil.

For carvone, all the bibliographic data reported below are from the IPCS-INCHEM evaluations (WHO Food Additive Series 28(1991), 42(1999) and FAO Nutrition Meetings Report Series 44a(1967).

B.6.5.1 Chronic toxicity and carcinogenicity study in rats

No data available

B.6.5.2 Chronic and carcinogenicity study in the mouse

Groups of 50 male and 50 female B6C3F1 (C57BL/6N, female x C3H/HeN MTV male) mice (7 week old) were administered 0, 375, or 750 mg/kg body weight d-carvone in corn oil by gavage, 5 days per week for 103 weeks. Animals were housed five per cage. Feed and water were available ad libitum. All animals were observed twice per day for clinical signs. No clinical signs were observed. Body weights were recorded once per week for the first 13 weeks of the study, and then at least once per month thereafter. The mean body weights of dosed and control male mice were similar throughout most of the study; the mean body weights of the dosed female mice were within 7% of those of the vehicle controls throughout most of the study. The survival of both the low dose (29/50 after week 101) and the high dose (38/50 after week 92) groups of female mice were significantly greater than that of the controls (14/50). This was probably caused by a very high death rate in the control females. The low survival of female vehicle control mice was related to a high incidence (52%) of ovarian abscesses similar to those seen in studies associated with Klebsiella sp. infections. Whether the lower incidence of ovarian abscesses in dosed female mice was related to d-carvone administration is uncertain. No differences in survival were observed between any groups of male mice. A necropsy was performed on all animals, including those found dead, except for tissues that were autolyzed. All organs and tissues were examined for grossly visible lesions and subjected to histopathology.

Several lesions occurred in the nasal cavities of male and female mice with dose-related increased incidences and/or severity. Atrophy of the olfactory epithelium and hyperplasia of the underlying Bowman's glands occurred together with high incidence in either sex in both dosed groups.

The effect on the nasal mucosa was probably due to a local effect of d-carvone caused by reflux of the gavage material when the gavage needle was withdrawn, since inflammatory exudate and evidence of the corn oil vehicle was found in over 50% of all dosed and control animals. No increases in tumour incidences were seen in mice administered d-carvone. The incidence of female control mice with primary neoplasms was 9/50 and the total number of primary neoplasms was 9. These numbers are low compared to 15/50 and 18 in the low dose female mice and 14/50 and 18 in the high dose mice and may be related to the early deaths of female control mice. The incidences of male mice with primary neoplasms (control, 27/50; low dose, 15/50; high dose, 16/50) and the total numbers of primary neoplasms (control, 38; low dose, 18; high dose, 20) were significantly lower in dosed groups than in vehicle controls. The lowered incidence of tumours in dosed male mice was primarily due to a lower incidence of subcutaneous fibromas, sarcomas, fibrosarcomas, or neurofibrosarcomas (NTP, 1989).

Special studies on carcinogenicity

Groups of female A/He mice were given intraperitoneal injections of d- or l-carvone three times weekly for eight weeks. The mice were then observed for a further 16 weeks until the end of the study. The total doses given of each compound were 1200 or 6000 mg/kg body weight. No increases in tumour incidences in the lung, liver, kidney, spleen, thymus, intestine, salivary or endocrine glands were observed. Approximately 20 and 40% of the d- and l-carvone treated animals, respectively, did not survive until the end of the study regardless of dose. Approximately 20% of the control animals died. The vehicle, tricaprylin, used to administer carvone was found to be impure and resulted in a weight loss of three to four grams in the control animals during the first week of the study (Stoner et al., 1973).

A group of approximately 15 female A/J mice was administered 0.2 mmol (approximately 30 mg) d-carvone by gavage one hour before oral administration of 20 mg/N-nitrosodiethylamine (NDEA)/kg body weight. This dosing schedule was repeated once a week for eight weeks and the experiment terminated 26 weeks after the first dose of NDEA. Forestomach tumour formation was significantly inhibited in d-carvone treated mice, with more than a 63% reduction in the mean number of papillomas per mouse compared to the controls. A significant reduction (34%) in the number of pulmonary adenomas per mouse was also observed in the d-carvone treated mice (Wattenberg et al., 1989).

Antimutagenic activity of water, chloroform and methanol extracts of Spearmint, has been demonstrated in rats (Yu et al (2004).

B.6.5.3 Summary and conclusions on long-term toxicity and carcinogenicity

Rats were exposed to 2500 ppm (approximately equivalent to 125 mg/kg bw/day) for one year without any observed adverse effects (study 2 under B6.3.1.1.). The RMS refrains from estimating the NOAEL to ≥ 125 mg/kg bw/day as we have too little information from this open literature study.

In a 24 week study l-carvone exposed mice showed decreased viability compared to d-carvone and control animals 60, 80 and 80 %, respectively). In another mouse study for 101/92 weeks (357/750 mg/kg bw) with d-carvone there was an indication that carvone protected against ovarian abscesses probably caused by infection. Two studies showed that there is no induction of tumours related to either d- or l-carvone. d-Carvone has also been shown to reduce the formation of tumours caused by N-nitrosodiethylamine. (A similar result has also been obtained with limonene.) Carvone can be considered as a non carcinogenic compound. No reliable NOAEL could be established as RMS only has access to summaries of studies taken from the open literature.

B.6.6 Reproductive toxicity (Annex IIA 5.6)

No available data, however, according to the notifier dietary exposure to Spearmint Oil at similar levels to calculated operator exposure has not resulted in reporting of any adverse reproductive effects in the general population.

B.6.7 Delayed neurotoxicity (Annex IIA 5.7)

No available data.

B.6.8 Further toxicological studies on carvone and limonene (Annex IIA 5.8)

As the active substance is a plant extract some extra information are needed e.g. microbiological quality of the harvest plants, detection and identification of possible contaminants such as heavy metals, toxins and pesticides. This information is given in Annex B.3.

B.6.8.1 Special studies on in vitro toxicity

All these studies were summarized by WHO [d-carvone and l-carvone-Food additives Series 28] as follows:

The toxicity of carvone (isomer not specified) to Ascites sarcoma BP8 cells (originating from inoculated C3H mice) cultured in suspension was examined. Carvone was incubated with the cells at 37°C for 48 hours at levels of 0.1 or 1.0 uM. The percent culture growth inhibition at these levels was 13 and 100%, respectively (Pilotti *et al.*, 1975).

The effect of carvone (isomer not specified) on cell metabolism was investigated by measuring the inhibition of noradrenaline- induced oxidative metabolism in isolated hamster brown fat cells. At a level of 1 mM carvone, there was a 42% inhibition of noradrenaline-induced oxidative metabolism (Pettersson *et al.*, 1980).

Carvone (isomer not specified) was reported to be ciliotoxic at a concentration of 5 mM in chicken tracheal organ cultures, with complete cessation of ciliary activity occurring six minutes after the addition of carvone to the culture (Pettersson *et al.*, 1982).

Carvone, (isomer not specified), at a concentration of 25 mM, was incubated for 30 minutes at 37 °C with labelled human lung fibroblasts. The degree of membrane damage, as measured by the release of an intracellular nucleotide marker, was significantly increased, with 95% of the nucleotides being released in 30 minutes (Thelestam *et al.*, 1980).

When carvone (isomer not specified) was incubated with microsomes from Phenobarbital pretreated rats a 25% conversion of cytochrome P-450 to cytochrome P-420 was observed. This conversion was not observed when rats were dosed (dose not specified) with carvone *in vivo* (Madyastha *et al.*, 1985).

The eggs of the insect *Earias vittella* were arranged in groups of 20 and exposed *in vitro* to various terpenoids. Appropriate controls were conducted and each experiment was replicated five times. Carvone (isomer not specified) did not inhibit embryonic development at any of the concentrations (not given) tested (Metha, 1979).

B.6.8.2 Observations in humans

No irritation was observed in humans exposed to concentrations of 1% l-carvone in petrolatum in a 48-hour closed patch test (Opdyke, 1973).

A maximization test was carried out on 25 volunteers. l-Carvone at a concentration of 1% in a petrolatum produced no sensitization reactions (Opdyke, 1973).

In one of 80 patients suffering from the condition of orofacial granulomatosis, characterized by swelling of the lips and lower half of the face, an association with intolerance to carvone (isomer not specified) was indicated from patch testing (Patton, *et al.*, 1985).

Three out of 33 patients tested for contact allergy to toothpaste flavours, experienced a positive allergic response in a patch-test using 5 % carvone (isomer not specified) in petrolatum (Anderson, 1978).

B.6.8.3 Other studies performed with limonene, see Appendix 1

As Spearmint Oil may contain a slight part of limonene, (4 % according to the five batches analyse but it can probably be higher in other batches) a presentation of the toxicological data on limonene was made in an Appendix. All the bibliographic data reported are from the IPCS-INCHEM evaluation (WHO Food Additive Series 30). See Appendix 1.

B.6.9 Medical data and information (Annex IIA 5.9)

No data are available on Spearmint Oil. However, data on carvone and limonene showed that the essential oil of *Mentha spicata* may induce dermal allergies.

B.6.9.1 Medicinal surveillance on manufacturing plant personnel: Medical examination of factory workers

No data available.

B.6.9.2 Report on clinical cases and poisoning incidents

No data available in the literature.

B.6.9.3 Observation on exposure of the general population and epidemiological studies if appropriate

In the safety evaluation of carvone prepared by WHO (series No 28, 42, FAO Nutrition Meetings Report Series 44a), per capita intake of carvone was estimated from data derived from surveys in Europe (International Organization of the Flavour Industry, 1995) and the United States (US National Academy of Sciences, 1987). The estimated total daily per capita intake of carvone (stereochemistry unspecified) and related substances from use as flavoring agents is 2.8 mg/person in Europe and 10 mg/person in the United States. Carvone accounts for approximately 99% of the total annual per capita intake of this group of substances when used as flavoring agents in Europe and 96% in the United States. Seven of the substances in this group of flavouring agents have been reported to occur naturally in foods, including fruits, spices, and berries.

l-Carvone or (-)-carvone (55-75%) has been reported in the oils of *Mentha* (spearmint).

d-Carvone or (+)-carvone (20-75%) has been reported in *Carum* (caraway) and *Anethum* (dill) (Maarse et al., 1994). Quantitative data on the natural occurrence have been reported for five of these substances; dihydrocarvone, dihydrocarvyl acetate, carvone, carveol and carvyl acetate (Nos 377, 379, 380, 381, and 382), which indicate that they are consumed predominantly in traditional foods (Stofberg & Kirschner, 1985; Stofberg & Grundschober, 1987 cited in WHO Food Additives Series No 42).

Numerous prospective and retrospective cohort epidemiological studies have been done on populations of refinery, chemical plant, and service station workers, exposed to mixtures of chemicals that have been associated with male rat $\alpha_2\mu$ -globulin nephropathy (Hanis *et al.*, 1979; Schottenfeld *et al.*, 1981; Hanis *et al.*, 1982; Thomas *et al.*, 1982; Austin & Schnatter, 1983; Higginson *et al.*, 1984; Raabe, 1984; Wen *et al.*, 1984; Wong & Raabe, 1989; Page & Mehlman, 1989). No statistically significant evidence was found in any study to link incidence of cancer of the kidney to work-place exposure to mixtures of complex hydrocarbons.

Igimi *et al.* (1976) reported that *d*-limonene was a safe and effective gallstone solubilizer in animals and humans; side effects in humans were reported to be pain and tenderness radiating from the upper abdomen to the anterior chest, nausea and vomiting, and diarrhoea.

B.6.9.4 Diagnosis of poisoning (determination of active substance, metabolites)

Cases Reported to the Company

No cases of poisoning have been reported to the company.

Literature

No data available in the literature.

B.6.9.5. Proposed treatment: first aid measures, antidotes, medical treatment

First Aid Measures

In case of ingestion, do not induce vomiting. Call a physician.

In case of inhalation, remove to fresh air. Call a physician.

Contaminated skin should be cleaned with soap and water; contaminated eyes should be rinsed with plenty of clean water for several minutes.

Antidotes, Medical Treatment

No specific antidote. Due to the low order of acute oral toxicity, antidotal treatment is not likely to be required. Supportive care. Treatment based on judgement of physician in response to symptoms of patient.

B.6.9.6. Expected effects of poisoning

No specific effects can be deduced from animal data. No human data are available.

B.6.10 Summary of mammalian toxicology and proposed ADI, AOEL, ARfD and drinking water limit (AnnexIIA 5.10)

A comparison between d- and l-carvone

The WHO Committee considered that optical enantiomers should not *per se* be regarded as toxicologically identical compounds, and therefore d-carvone and l-carvone should be evaluated separately. Although both compounds were discussed on a meeting, there were only sufficient data for toxicological evaluation for

d-carvone. One concluded that rats appeared to be more susceptible to d-carvone than mice.

As the toxicological data are mostly limited to d-carvone, and Spearmint Oil contains around 80 % l-carvone the RMS has tried to find how similar or different biological effects the two enantiomers have. The RMS has found the following differences between l-carvone and d-carvone:

In a mouse cancer study the viability after 24 weeks were 80% in control and d-carvone exposed animals but 60% in l-carvone exposed mice (Stoner et al 1973) indicating a slightly greater toxic potential of l-carvone. No significant increases in tumour incidences in the lung, liver, kidney, spleen, thymus, intestine, salivary or endocrine glands were observed as a result of exposure to either d- or l-carvone (although there was a higher tumour incidence in d-carvone treated mice than in l-carvone treated mice). De Sousa et al 2007 found that the LD₅₀ value in mice was 427 mg/kg for l-carvone and 484 mg/kg for d-carvone.

In the central nervous system the two isomers sometimes react similar and sometimes differently: Both d-carvone and l-carvone have depressant effects in the CNS.

Pre-treatment of mice with d-carvone could increase the latency of convulsions, close to that of a standard anticonvulsant drug, whereas l-carvone had no effect on the onset of convulsions. The results show that the chiral center at carbon 4 in the carvone molecule is important in the interaction with receptors. The author suggests that carvones possibly interact with GABA_A receptors in the brain after crossing the blood-brain barrier (De Sousa et al. 2007).

l-Carvone showed more sedative and relaxant effects after inhalation, whereas d-carvone demonstrated both sedation and activation effects on the locomotion of mice. Buchbauer et al 2005.

The overall conclusion is that l-carvone does not seem to be more toxic than d-carvone.

B.6.10.1 Absorption, distribution, excretion and metabolism (toxicokinetics)

The data provide no information on the percentage absorption after oral, dermal or inhalation exposure, the distribution of l-carvone (the main component in Spearmint Oil) or the rate and routes of elimination from the body. Therefore, default values of 50, 100 and 100% for oral, dermal and inhalation absorption respectively, will be used for the exposure and risk assessment calculations for operators, workers and bystanders.

B.6.10.2 Acute studies

Table B.6.10.2 – 1: Overview of acute toxicity studies with HUILE DE MENTHE (Spearment Oil), containing 74% l-carvone

Parameter	Species	Result	Reference
Oral LD ₅₀	Rat	>2 000 mg kg ⁻¹	Mullaney, 2005
Dermal LD ₅₀	Rat	>2 000 mg kg ⁻¹	Mullaney, 2005
Inhalation 4 hr LC ₅₀	Rat	>5.43 mg/L	Griffiths and Watson, 2006
Skin irritation	Rabbit	Non-irritant	Mullaney, 2005
Eye irritation	Rabbit	Mild irritant	Mullaney, 2005
Skin sensitisation	Mouse	Sensitizer	Mullaney, 2005

The acute oral, dermal, and inhalation toxicity tests were performed in one species, rat. The ataxia, seen in the oral and inhalation limit tests only, indicates that part of the nervous system was temporarily affected. This finding is supported by a study by Gordon et al, 1982 (in the safety evaluation of carvone prepared by WHO (Food Additive Series No. 28) where d-carvone was administered by intraperitoneal injection to mice in graded doses (not given) up to 800 mg/kg body weight at which high dose level significant convulsive effects occurred.

Based on the above results, HUILE DE MENTHE/Spearment Oil does not need to be classified with respect to its acute oral, dermal and inhalation toxicity. Nevertheless, as HUILE DE MENTHE/Spearment Oil is composed of alicyclic hydrocarbons, has a kinematic viscosity of 2.95×10^{-6} m²/sec at 20°C and has a surface tension of 31.13 mN/m, it does present a theoretical aspiration hazard to humans; on this basis it meets the criteria for assignment of the symbol “Xn”, indication of danger harmful and the risk phrase R 65 Harmful: may cause lung damage if swallowed. HUILE DE MENTHE/Spearment Oil did not meet the criteria for classification as irritant to skin or eyes according to EU labelling regulation. HUILE DE MENTHE/Spearment Oil was a skin sensitizer in the animals tested and the test material was classified as a sensitizer according to EU labelling regulations. The symbol “Xi”, indication of danger irritant and the risk phrase R 43 “May cause sensitisation by skin contact” are therefore required.

B.6.10.3 Complementary information of acute toxicity of carvone cited in WHO Food Additive Series 28, 42, FAO Nutrition Meetings Report Series 44a

Table 5.2.1.2: Summary of acute toxicity studies published on carvone

Species	Route	LD ₅₀	Purity	reference
Mouse	S.C.	2675 mg/kg bw	NS	Wenzel & Ross, 1957
Rat	Oral	1640 mg/kg bw ^a	NS	Jenner et al., 1964
Rat	Oral	3710 mg/kg bw ^b	NS	Opdyke, 1978
Guinea-pig	Oral	766 mg/kg bw ^c	NS	Jenner et al, 1964
Rabbit	Dermal	3860 mg/kg bw	NS	Opdyke, 1978

S.C = Subcutaneous. NS = Not stated. N = No. ^a Oral LD₅₀ reported on "carvone", identified as p-mentha-6,8-dien-2-one. ^b Oral LD₅₀ reported on d-carvone. ^c Dermal LD₅₀ reported as 4 ml/kg body weight for d-carvone.

Single intraperitoneal injections of d-carvone to male Swiss- Webster albino mice did not result in any observed hepatotoxic or pulmonary effects (Gordon et al, 1982)..

B.6.10.3.1 Short-term toxicity

No short term toxicity study results are available with Spearmint Oil. However, as the main component of Spearmint Oil is l-carvone and for a little part limonene, this part includes the bibliographic data on both these components, as representative of the toxicity of the essential oil. (Limonene data see B.6.8). The RMS has estimated NOAELs from the results that are available in these bibliographic documents. Note that the NOAELs are not based on the original studies!

B.6.10.3.1.1 Oral

Table B.6.10.1.1 – 1: Summary and conclusions on oral short-term toxicity studies on carvone

Animals	Isomer	Dose level	Duration time	NOEL/NOAEL*	Organ effects	Ref
<i>Oral administration in rat</i>						
5 male + 5 female	Not specified	50 mg/kg bw/day	27-28 weeks	NOAEL > 50 mg/kg bw/day	No adverse effects judged by growth, appearance, food intake, haematology, final body weights, or gross and microscopic examination of the major organs	Hagan et al., 1967
5 male + 5 female	Not specified	125 mg/kg bw/day	1 year	NOAEL > 125 mg/kg bw/day	No adverse effects judged by growth, appearance, food intake, haematology, final body weights, or gross and microscopic examination of the major organs	Hagan et al., 1967
5 male + 5 female	Not specified	500 mg/kg bw/day	16 weeks	NOAEL < 500 mg/kg bw/day	Growth depression and testicular atrophy	Hagan et al., 1967
5 male + 5 female	d-carvone	0, 150, 328, 723, 1590, 3500 mg/kg bw/day	5 days per week, for 12 doses over 16 days	NOAEL 150 mg/kg bw/day	Increase in kidney weight at 328 mg/kg bw/day followed by decrease in thymus and brain weight at 723 mg/kg bw/day. All rats receiving 1590 and 3500 mg/kg bw/day died.	NTP, 1989
40 males + 20 females	d-carvone	0, 93, 187, 375, 750, 1500 mg/kg bw/day	5 days per week, for 13 weeks	NOAEL < 93 mg/kg bw/day	Decreased body weight, increased relative kidney and liver weight at 93 mg/kg bw/day, testis and lungs weights in male at 187 mg/kg bw/day, testinal degradation at 750 mg/kg bw/day. All rats receiving 1500 mg/kg bw/day died.	NTP, 1989
<i>Oral administration in mouse</i>						
5 animals/ group and sex	d-carvone	0, 150, 328, 723, 1590, 3500 mg/kg bw/day	5 days per week, for 12 doses over 16 days	NOAEL < 150 mg/kg bw/day	Increased liver weight in male and decreased thymus weight in female at 150 mg/kg bw/day	NTP, 1989
40 males + 20 females	d-carvone	0, 93, 187, 375, 750, 1500 mg/kg bw/day	5 days per week, for 13 weeks	NOAEL 375 mg/kg bw/day	Increase in liver weight at 750 mg/kg bw/day. Most of the animals died at 1500 mg/kg bw/day	NTP, 1989

* Note that this is an attempt to roughly estimate NOAELs as RMS only has had access to summaries of studies from the open literature!

The oral short-term toxicity for carvone has been studied in rat and mouse. Note that no studies have been performed with Spearmint Oil or pure l-carvone and that RMS only has had access to studies from the open literature! This makes the estimated NOAELs rather rough. The major detected adverse effects were growth depression, testicular atrophy, and increase in liver and kidney weight and decreased thymus weight. No reliable NOAEL can be established but taken the available studies together, the rat seems to be a little more sensitive than the mouse and the NOAEL seems to be in the range of 50 to 100 mg/kg bw /day based on decreased body weight and increased relative liver and kidney weight.

B.6.10.3.1.2 Inhalation

There are no studies of Spearmint Oil or carvone.

B.6.10.3.1.3 Dermal

There are no studies of Spearmint Oil or carvone.

Dermal application of d-limonene has been reported to cause irritation and immune-mediated skin reactions in a number of species, including humans.

B.6.10.3.1.4 Genotoxicity

There are no available data on Spearmint Oil or l-carvone. From one study it was concluded that d-carvone did not have any mutagenic activity in the bacteria test system. The results from the in vivo studies with d-carvone looking for sister chromatid exchange and chromosomal aberrations were equivocal and no conclusions could be drawn.

In 11 different studies limonene did not show any genotoxic effects in any cell system either.

B.6.10.3.2 Long-term toxicity and carcinogenicity

In a study to examine the toxicity of a large number of flavouring agents, groups of five male and five female Osborne-Mendel weanling rats were fed a diet containing carvone at a concentration of 2500 mg/kg (equivalent to 125 mg/kg bw per day) for one year. The stereochemistry of the test material was unspecified, but according to the notifier, a survey of industrial producers of carvone who actively marketed carvone during the period of the study (1960-70) indicated that the material in commerce in the United States at that time was the l-carvone (Bauer, 1991; Flynn, 1991; Wrigley Co., 1991). (The RMS have not been able to verify this statement). Body weights and food intake were measured weekly and haematological examinations performed at 3, 6, 12, and 22 months. Depressed body-weight gain and testicular atrophy at a dose of 750 mg/kg bw per day were the reported effects. The NOEL was 125 mg/kg bw per day (Hagan et al., 1967).

In a 24 week study l-carvone exposed mice showed decreased viability compared to d-carvone and control animals 60, 80 and 80 %, respectively). In another mouse study for 101/92 weeks (357/750 mg/kg bw) with d-carvone there was an indication that carvone protected against ovarian abscesses probably caused by infection. Two studies showed that there is no induction of tumours related to either d- or l-carvone. d-Carvone has also been shown to reduce the formation of tumours caused by N-nitrosodiethylamine. (A similar result has also been obtained with limonene.) Carvone can be considered as a non carcinogenic compound.

No reliable NOAEL could be established as RMS only has access to summaries of studies taken from the open literature.

B.6.10.3.3 Reproduction toxicity

No studies are available on Spearmint Oil or carvone, however, dietary exposure to Spearmint Oil at similar levels to calculated operator exposure has not resulted in reporting of any adverse reproductive effects in the general population.

Results of teratogenicity studies in mice suggest that maternal consumption of 2400 mg of d-limonene/kg bw/day but not of 600 mg/kg bw/day, affected the development of fetuses (decreased body weight gain, increased incidences of lumbar rib and fused rib, and delayed ossification). However, d-limonene was reported to be non-teratogenic in rabbits at doses ranging from 250 to 1000 mg/kg bw/day (although the highest dose significantly reduced survival of the dams).

B.6.10.3.4 Delayed neurotoxicity

No studies are available on Spearmint Oil or carvone.

B.6.10.4 Acceptable Daily Intake (ADI)

Spearmint Oil is used in many different food products. The following document is from Fenaroli's Handbook of Flavor ingredients, volume 1, 3rd ed, CRC press 1995, ISBN 0-8493-2710-5. Spearmint Oil level of use is what the industries in US has reported that they use in different products.

SPEARMINT OIL
Annex B.6: Toxicology and Metabolism

SPEARMINT

Botanical source	<i>Mentha spicata</i> Houds or L.		
Botanical family	Labiatae		
Foreign names	Menthe crépue (Fr.), Krauseminz (Ger.), Menta cressa (Sp.), Menta crispa (It.)		
Description	Herbaceous plant extensively cultivated in North America, England, Germany, and Holland. The most frequently cultivated varieties are <i>M. trichoura</i> Brig., <i>M. crispata</i> , and <i>M. tenuis</i> . The plant is from 25 to 75 cm (10 to 30 in.) high, with an erect, branched stalk exhibiting long, lanceolate leaves, and flowers clustered in the form of spikes.		
Parts used	Flowering tops		
Physical-chemical characteristics			
Essential oil	The oil, obtained by steam distillation, is a pale yellow to greenish-yellow liquid with an aromatic odor. The oil contains α -pinene, α -phellandrene, <i>l</i> -limonene, octyl alcohol, dipentene cineol (in some varieties only), dihydrocarveol, and carvone.		
	Specific gravity at 20°C	0.915 to 0.938 ¹	
	Refractive index at 20°C	1.4830 to 1.4910 ¹	
	Optical rotation	- 40° to - 60°C ¹	
	Ketone content (as carvone)	55 to 71.5% ¹	
	Solubility	1:1 in 80% ethanol ¹	
Organoleptic characteristics	Warm, herbaceous, penetrating odor; sharp, pungent flavor		
Reported uses ³			
	Spearmint		
	Baked goods	5967. ppm	
	Meat products	804.8 ppm	
	Gelatin, pudding	2000. ppm	
	Nonalcoholic beverages	1674. ppm	
	Alcoholic beverages	500.0 ppm	
	Spearmint extract		
	Nonalcoholic beverages	2000. ppm	
	Alcoholic beverages	2000. ppm	
	Spearmint oil		
	Baked goods	1318. ppm	
	Frozen dairy	130.4 ppm	
	Fruit juice	550.0 ppm	
	Condiment, relish	250.0 ppm	
	Soft candy	559.6 ppm	
	Jam, jelly	98.66 ppm	
	Sweet sauce	90.00 ppm	
	Gelatin, pudding	95.42 ppm	
	Nonalcoholic beverages	136.0 ppm	
	Alcoholic beverages	154.3 ppm	
	Hard candy	1605. ppm	
	Chewing gum	7913. ppm	
	Seasonings, flavors	66668 ppm	
Regulatory status	<i>Spearmint</i> : CFR 182.10, 582.10; FEMA No. 3030		
	<i>Spearmint extract</i> : CFR 182.20, 582.20; FEMA No. 3031		
	<i>Spearmint oil</i> : CFR 182.20, 582.20; FEMA No. 3032		

The Notifier has made a calculation of how much anti sprouting treatment with Spearmint Oil would contribute to the already well known daily intake of Spearmint Oil. (See the following discussion paper received by RMS 2007-01-30.) The calculation is a worst case scenario.

The calculation relay on the fact that in US the daily intake of Spearmint Oil is 0.458g/kg bw/day. That correlates to an intake of pure l-carvone of 0.252 mg/kg bw/day. If the residues level of l-carvone is 1.84 mg/kg that would give a maximum intake of l-carvone of 0.006 mg/kg bw/day from anti sprouting treated potatoes. In a 28 weeks rat study, the lowest effect level with carvone was 93 mg/kg bw/day and in one 13 weeks study no effects was seen with 50 mg/kg bw/ day. If an intake of l-carvone of 0.006 from eating anti sprouting treated potatoes is compared to the no effect level in the rat study it is a value close to 10 000 times lower. This cannot give rise to any risk for the consumer, as the margin of safety is enough.

B.6.10.4.1 Theoretical maximum daily intake – Discussion paper from the notifier

DISCUSSION PAPER
Spearmint Oil / L-Carvone :
Normal Dietary Exposure
Versus
Potato Anti-Sprouting Use Additional Exposure

It is important to recognise that the safety evaluation of an added use of a substance needs to be conducted with an appreciation of the overall exposure. If the added use is trivial in terms of its contribution to overall exposure, this fact needs to be taken into consideration.

Excluding use of Spearmint Oil as a potato sprout suppressant, a theoretical daily dietary intake for L-Carvone originating from Spearmint Oil has been calculated. A **minimum** 55% w/w content of L-Carvone in Spearmint Oil has been assumed for this calculation (Coëffic, M (2007)). For Spearmint Oil consumption estimation, no suitable EU or World Health Organization (WHO) data have been identified, however, data from the USA are considered to be of surrogate value. For the entire US population, Spearmint Oil consumption is 0.000458 g/kg bw/day or 0.458 mg/kg bw/day (Anon. (1997)). The **minimum** L-Carvone content of 0.458 mg Spearmint Oil can be calculated as $0.55 \times 0.458 = 0.252$ mg. The corresponding **minimum** L-Carvone dietary consumption is 0.252 mg/kg bw/day.

For comparison, a Theoretical Maximum Daily Intake (TMDI) for L-Carvone **resulting from use of Spearmint Oil as a sprout suppressant on potatoes** has been calculated. For potato consumption estimation, data from the WHO European Regional Diet have been utilised. European potato consumption is estimated at 240.8 g/person/day (Anon. (2003)). The **worst case** L-Carvone residue resulting after use of Spearmint Oil as a sprout suppressant in potatoes according to Good Agricultural Practice has been reported as 1.84 mg/kg (Ruffet, M-C. (2005)). Consequently a **worst case** dietary exposure to L-Carvone from this use can be calculated as $1.84 \times 0.2408 = 0.443$ mg/person/day or for a 70 kg adult $0.443/70 = 0.006$ mg/kg bw/day.

Comparing the TMDI of L-Carvone **resulting from use of Spearmint Oil as a sprout suppressant on potatoes**, 0.006 mg/kg bw/day, with the theoretical **minimum** daily dietary intake of L-Carvone **excluding use of Spearmint Oil as a sprout suppressant**, 0.252 mg/kg bw/day, it can be calculated that even in this **worst case** assessment, the former represents only 2.4% of the latter. This is a trivial increase in total dietary exposure to L-Carvone, and almost certainly within the limits of likely variation in dietary consumption of Spearmint Oil. In reality, the increase in total dietary exposure to L-Carvone will be even more trivial than calculated above, as not all potatoes will be treated post-harvest with Spearmint Oil.

Although this exposure assessment focuses on L-Carvone as a marker residue for Spearmint Oil, this form of assessment has the advantage that it is holistic in nature, being indicative of dietary exposure to (all of the components in) the active substance Spearmint Oil (the whole oil is regarded as the active substance).

is of this document.

WARNING: This r

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B.6.10.5 Parametric value for Drinking water

As the limit in the Drinking Water Directive is 0.1 µg/L, no other value will be proposed

B.6.10.6 Acceptable Operator Exposure level (AOEL)

Normally an AOEL can be established using a relevant NOAEL from the toxicological animal studies and safety factors. In this case there are no relevant NOAELs available for Spearmint Oil/l-carvone so instead the RMS has estimated the exposure for operators and compared it to the known daily intake of Spearmint Oil/carvone from food.

The operator working with Spearmint Oil is only exposed during loading the hot fogging apparatus. The hot fogging treatment is a closed system and the operator is not exposed. According to the calculation made by the notifier the operator using PPE is exposed to 0.23 mg carvone/kg bw/day which is in the same range as the estimated daily intake of carvone in the US (0.252 mg/kg bw/day). The operator will not be exposed daily as the hot fogging treatment will only be repeated once every third week. Comparing this to the likely normal variation in carvone intake from food, **the RMS considers this exposure as not being a risk.**

B.6.10.7 Acute reference Dose (ARfD)

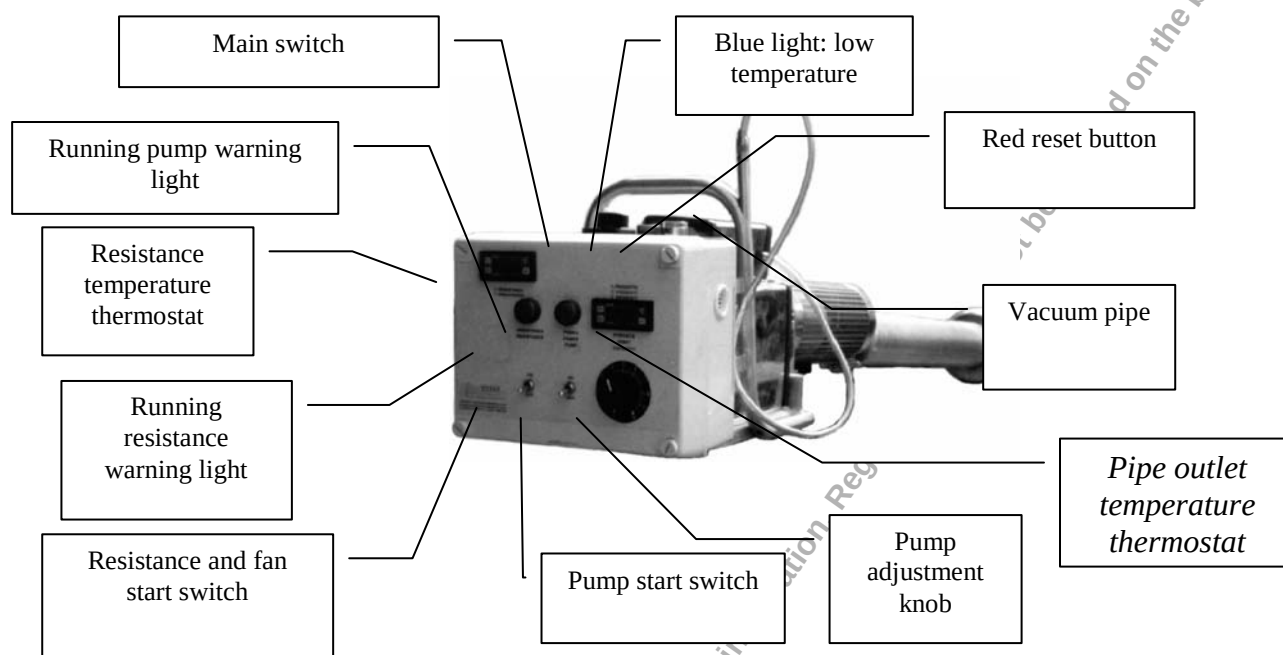
The assignment of an ARfD is not considered required due to the low acute toxic potential of Spearmint Oil/l-carvone, which is a normal constituent of the human diet.

B.6.10.8 Estimation of operator exposure

Normally an AOEL can be established using a relevant NOAEL from the toxicological animal studies and safety factors. In this case there are no relevant NOAELs available for Spearmint Oil/l-carvone so instead the RMS has estimated the exposure for operators, bystanders and workers and compared it to the known daily intake of Spearmint Oil/carvone from food.

Low exposure of operators during loading of neat Spearmint Oil into proprietary Xeda Electrofog hot fogging equipment (see below) is facilitated by the engineering controlled vacuum pipe filling technique and associated use of appropriate Personal Protective Equipment (PPE), including gloves, goggles and safety shoes.

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Exposure to the major component of Spearmint Oil, l-carvone, during loading has been estimated assuming: a 75% l-carvone content in Spearmint Oil; a 5 L container size (volume of hand contamination is estimated as 0.01 mL/operation, an overestimation for filling fogging apparatus according to Section 4.4 of the CTB's chlorpropham evaluation at http://www.ctb.agro.nl/ctb_files/05829_12.html); a work rate of one 1000-tonne store facility treated per day (maximum volume allowing quick loading of crop in uniform condition); an organic solvent formulation, i.e. neat Spearmint Oil (nominal transfer of concentrate from protective gloves to hands is estimated as 10%); high default percutaneous absorption (100%); and 18 operations per day ((a maximum 90 g/tonne application rate x 1000 tonnes store capacity)/5000 g container size). When applying Spearmint Oil, no significant exposure of operators is expected, as hot fogging application is engineering controlled from outside of the storage facility via treatment ports (see illustration of a built-in treatment port below). This is essentially a remotely controlled indoor application technique, which in addition to being protective of the operator and any bystanders, is also environmentally protective.

See Appendix 2 with UK Pesticides Safety Directorate Predictive Operator Exposure Model with incorporation of the above mentioned data and estimation of operator exposure. The operator was exposed to 0.23 mg l-carvone /kg bw/day which is in the same range as the estimated daily intake of carvone in the US (0.252 mg/kg bw/day). **The RMS considers this exposure to not be a risk.**

B.6.10.9 Estimation of bystanders exposure

During the application of the product no un-authorized person should stay in or close to the application area.

B.6.10.10 Estimation of workers exposure

It is not normally necessary that treated potatoes are handled prior to minimisation of residues of the applied product; however personal protective equipment including gloves, goggles/faceshield and organic vapours protective mask, must be used if workers need to enter into the treatment silo during at least the first 24 hours following the end of the treatment.

According to point 4.4.3 Worker Exposure/Risk of the CTB evaluation of chlorpropham at

http://www.ctb.agro.nl/ctb_files/05829_12.html: "There is no model available to estimate exposure at re-entry of potato storages after fogging. However, a qualitative risk assessment is possible. This is based on the instructions for intended use on the label. According to the label, internal ventilation has to be done till the fog has completely precipitated. The storage has to be kept closed for another 24 hours. Thereafter, ventilation as usual is advised. In practice re-entry shortly after this procedure will be only for control and maintenance activities with little contact to treated potatoes. Fogging will not be done shortly before emptying the storage. This reduces exposure to chlorpropham at re-entry to minimal levels."

A similar case to that above can be made for Spearmint Oil and l-carvone (although ventilation as usual begins 2 hours, rather than 24 hours, after treatment, facilitating an earlier reduction in any potential exposure). It is of additional note that Spearmint Oil is not classifiable with respect to acute inhalation toxicity (rat 4h nose only LC50 > 5.43 mg/L mean achieved atmosphere concentration (nominal concentration 18.7 mg/L); no mortalities at this high dose level, and full short-term recovery from clinical signs; Griffiths & Watson 2006).

Low exposure of workers handling Spearmint Oil treated potatoes can be ensured by the low residues on potatoes when collected from storage three weeks post-application (maximum 1.84 mg l-carvone/kg potato), combined with PPE in the form of gloves which are necessary to minimize risk of skin sensitisation. For estimation of post-treatment exposure of workers during potato sorting, packing and culling operations, the following assumptions have been made (approach based on US EPA assessment of post-treatment exposure of workers during citrus sorting, packing and culling operations): a medium ware (food) potato has a diameter of ca. 6.25 cm (for the purposes of this calculation potatoes are assumed to be roughly spherical) and weighs 216 grams (UK PSD Commodity size data for whole raw commodities); the maximum measured l-carvone residue level in potatoes treated by hot fogging is 1.84 ppm (µg/g) after 3 weeks; essentially all of the l-carvone residue in a treated potato is located on the surface (the chemical is not applied as a systemic product); the surface area of the palmar surface of the hands is 410 cm² (this area is considered to be the total area exposed in an 8 hour day); a study from the scientific literature (Brouwer et.al. 1999., Appl. Occ. Environ. Hyg 14, 231-239) indicates that less than 2 per cent of material from glass plates treated with a dust is transferred to the hands after repeated pressing (this is assumed to be similar to grasping potatoes). The amount of l-carvone in a potato = the measured residue of 1.84 µg/g x the potato mass of 216 g = 397.5 µg. The surface area of a 6.25 cm diameter sphere = $4 \times \pi \times r^2 = 4 \times 3.14 \times (6.25/2)^2 = 122.7 \text{ cm}^2$. The surface residue for l-carvone would therefore = $397.5 \text{ µg} / 122.7 \text{ cm}^2 = 3.24 \text{ µg/cm}^2$. If 2 percent of the material on a surface transfers to the hands, the dermal exposure = surface residue x palmar surface of hand exposed/day x 2% ÷ 70 kg = $3.24 \text{ µg/cm}^2 \times 410 \text{ cm}^2/\text{day} \times 0.02 \div 70 \text{ kg} = 0.4 \text{ µg/kg bw/day} = 0.0004 \text{ mg/kg bw/day}$.

Working with anti sprouting potatoes will then expose the worker with 0.0004 mg/kg bw/day which is around 0.09% of the daily carvone intake from food in the US (0.252 mg/kg bw/day). **The RMS does not considering this being a risk.**

The unprotected worker dermal exposure scenario described above is considered to be conservative for the following reasons: the transfer coefficients for the hand presses were obtained from a study in which a dust formulation was transferred from glass plates, rather than from a study in which a liquid was removed from a dry surface such as in a conveyer belt or potato skin scenario; the exposure estimates were not corrected for dermal absorption (dermal absorption is assumed to be 100%); contact with clean surfaces removes some of the material (such contact is not considered in the estimates). Also, in practice, PPE in the form of gloves is worn by workers handling treated potatoes, and the resulting dermal exposure is thereby decreased by a factor of 10 compared to the unprotected scenario.

B.6.11 Acute toxicity including irritancy and skin sensitization of preparations (Annex IIIA 7.1)

As the preparation and the active substance is the same see B.6.2.

B.6.12 Dermal absorption (Annex IIIA 7.3)

As the preparation and the active substance is the same see B.6.1.

B.6.13 Toxicological data on non active substances (Annex IIIA 7.4 and point 4 of the introduction)

Not applicable.

B.6.14 Exposure data (Annex IIIA 7.2)

See Appendix 2

B.6.15 References relied on

SPEARMINT OIL

Mentha piperita L. var. piperita L.

Dossier file No.	Author(s)	Year	Title Source Company report no. GLP / GEP status Published	Data Protection Y/N*	Owner
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IIA 5.2	DR Griffiths and P Watson	2006	Huile de menthe hn: Acut inhalation toxicity (nose only) study in the rat. [REDACTED] project no 1620/045. GLP yes, unpublished	Y	XEDA International
IIA 5.2	T Mullaney	2005	Huile de menthe hn: Acut dermal irritation in the rabbit. [REDACTED] project no 1620/046. GLP yes, unpublished	Y	XEDA International
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IIA 5	FAO	1967	WHO Food Additives Series No 44a - International programme on chemical safety. FAO Nutrition meetings. Resort Series No 44a. Toxicological evaluation of some flavouring substances and non-nutritive sweetening agents. d-carvone and l-carvone, Geneva, 1967	N	Published paper
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IIA 5	WHO	1993	WHO Food Additives Series No 30 - International programme on chemical safety - Limonene - First draft prepared by Dr K.B. Ekelman and Dr D. Benz. US Food and Drug Administration, Washington DC, USA.	N	Published paper
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* Protection for 5 years claimed from date of decision concerning listing in Annex I - the study report has not been submitted to any of the Member States in support of an application for authorization, or (though the study report has been submitted) has not been used in any of the Member States as the basis for decision on the initial authorization, or to maintain a given authorization of a plant protection product before the date of submission of the dossier to the Rapporteur Member State.

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<http://emt.oregonstate.edu/faculty/pdfs/dashwood/dashwoodAntimutagenic.pdf>

APPENDIX 1 - Limonene

As Spearmint Oil may contain a slight part of limonene (4 % according to the five batches analyse but it can probably be higher in other batches) a presentation of the toxicological data on limonene was made in an Appendix. All the bibliographic data reported are from the IPCS-INCHEM evaluation (WHO Food Additive Series 30).

Absorption, distribution, metabolism and excretion of limonene

d-Limonene was rapidly absorbed (43 min) through the intact, shaved abdominal skin of mice (Meyer & Meyer, 1959).

Twelve Long-Evans male rats were administered single topical doses of 5 mg/kg bw ¹⁴C-limonene; the treated area was then occluded for 3 h (2 males) or 6 h (10 males). Following occlusion, the residual dose was removed and the treated area was re-occluded. Pairs of treated rats were killed at 3, 6, 24, 48, and 72 h; urine and faeces were collected from rats killed at 24, 48, and 72 h, and plasma and tissue samples were taken at all time points. Authors reported that peak concentrations of radioactivity in tissue samples were measured 3-6 h after dosing in the gastrointestinal tract (0.1-0.4% dose/g), livers and kidneys (0.08-0.2% dose/g), and thyroid and fat (0.02-0.06% dose/g); except for the gastrointestinal tract, concentrations of radioactivity in all tissues were appreciably lower at 24 h. After 6 h of exposure, 48% of the radioactivity was recovered in the skin; at the 24-72 h sampling times, 8-12% was excreted in urine, 1-3% was excreted in faeces, and 14-18% was expired in air. Total mean recovery of radioactivity was reported to be approximately 76%.

Following oral administration, the highest concentration of d-limonene or its metabolites in rats was found in the serum fraction of blood after 2 h. The other major organs containing metabolites of d-limonene were the liver and kidney, with peaks 1-2 h after ingestion. After 48 h, negligible amounts of d-limonene metabolites remained in the body. Approximately 60% of d-limonene was excreted in the urine, 5% in the faeces, and 2% was expired (Igimi et al., 1974).

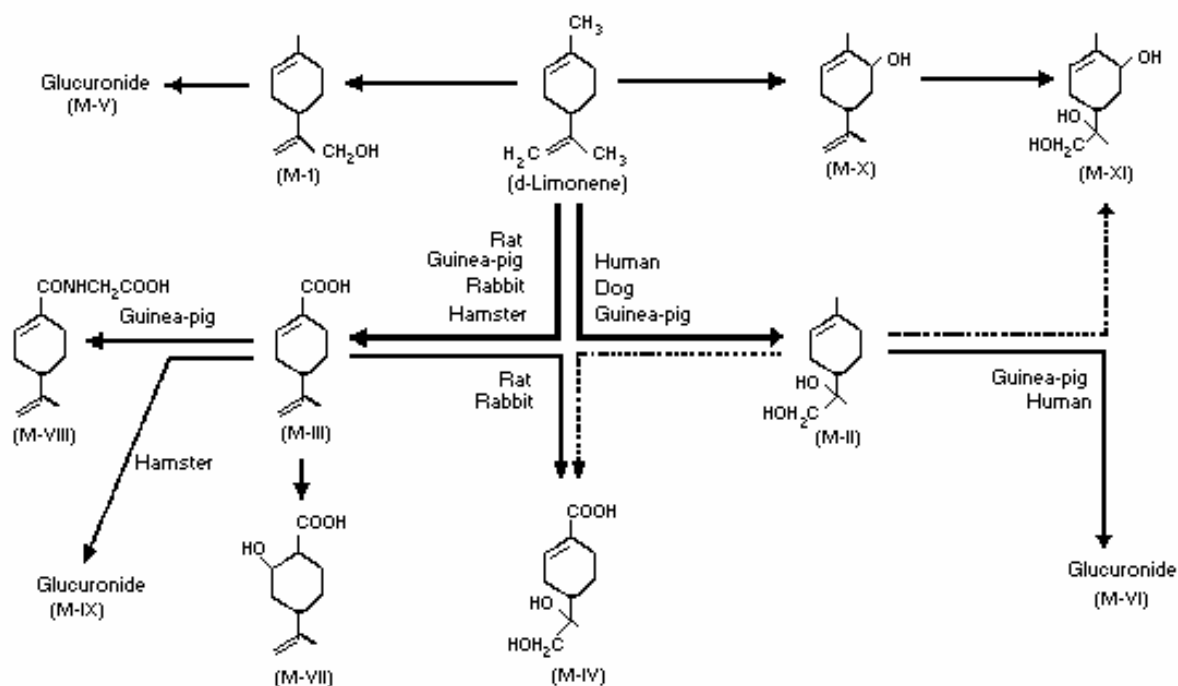
Kodama et al. (1974) reported that 72% of d-limonene metabolites were excreted in male rabbit urine 72 h after oral administration, while 7% was found in the faeces.

Biotransformation of limonene

Kodama et al. (1974) identified the metabolites of orally administered d-limonene (also known as p-mentha-1,8-diene) in male rabbit urine as p-mentha-1,8-dien-10-ol (M-I), p-menth-1-ene-8,9-diol (M-II), perillic acid (M-III), perillic acid 8,9-diol (M-IV), p-mentha-1,8-dien-10-yl-beta-D-glucopyranosiduronic acid (M-V) and 8-hydroxy-p-menth-1-en-9-yl-beta-D-glucopyranosiduronic acid (M-VI).

The list of metabolites was expanded based on a study done with male rats, hamsters, guinea-pigs, rabbits, dogs and humans (Kodama et al., 1976b). Five additional metabolites of d-limonene were identified: 2-hydroxy-p-menth-8-en-7-oic acid (M-VII), perillylglycine (M-VIII), perillyl-beta-D-glucopyrano-siduronic acid (M-IX), p-mentha-1,8-dien-6-ol (M-X) and p-menth-1-ene-6,8,9-triol (M-XI). The major metabolite in rats and rabbits was identified as M-IV, while the major metabolite in hamsters was found to be M-IX. In dogs, the major metabolite was M-II, but in guinea pigs and humans, it was M-VI. The possible metabolic pathways used by the various species are shown in Figure 1 below.

Figure 1. Major pathways for d-Limonene Metabolism



Wade et al. (1966) reported that dietary limonene gives rise to uroterpenol (M-II: p-menth-1-ene-8,9-diol) in the urine of humans.

Effects limonene on enzymes and other biochemical parameters

Groups of control (6 animals) and experimental (4 animals) male Wistar rats were administered single gavage doses of 0, 200, 400, 600, 800, or 1200 mg/kg bw d-limonene in 2% tragacanth solution in a total volume of 4 ml/kg. Authors reported that no effects were observed on liver triglycerides, microsomal proteins, cytochrome B5 and drug metabolizing enzymes.

In the same experiment, male Wistar rats were treated with gavage doses of 0 or 400 mg/kg bw d-limonene in 2% tragacanth solution in a total volume of 4 ml/kg for 2, 3, 15 or 30 days; animals were killed 24 h following the last dose. Authors reported that, following repeated treatment for 30 days, relative liver weight and hepatic phospholipid content were slightly increased, and liver and serum cholesterol were decreased 49% and 8%,

respectively. In addition, palmitic, linoleic and arachidonic acids were increased, and stearic acid was decreased in the liver; aminopyrine demethylase and aniline hydroxylase were increased 26% and 22%, respectively, and cytochrome P-450 and b5 were increased by 31% and 30%, respectively (Ariyoshi et al., 1975).

d-Limonene was reported to enhance bile flow in Wistar rats and mongrel dogs in a dose-related manner, and to decrease the ratio of biliary bile salts and phospholipids to cholesterol (Kodama et al., 1976). d-Limonene was reported to be an effective gallstone solubilizer in animals and humans (Igimi et al., 1976; Schenk et al., 1980), and to have no effect on liver weights or levels of serum lipids when fed to male Wistar rats at 0.5 and 1% in the diet (Imaizumi et al., 1985).

d-Limonene was reported to significantly damage and increase permeability of membranes of human lung fibroblasts (Thelestam et al., 1980; Curvall et al., 1984).

Acute toxicity of limonene

For limonene, all the bibliographic data reported below are derived from the IPCS-INCHEM evaluation (WHO Food Additive Series 30).

An oral dose of 3 ml of d-limonene (in juice)/kg decreased spontaneous motor activities in rats and mice and potentiated hexobarbital-induced sleeping and hypothermia in mice. Authors also reported that nicotine-induced convulsion and death (but not maximum electroshock-, pentetrazol-, strychnine-, nor picrotoxin-induced convulsions) were inhibited by d-limonene in mice. Intravenous administration of > 0.005 mg/kg d-limonene lowered the blood pressure of rabbits and dogs, and > 0.1-0.3 mg/kg killed those animals. Oral administration of 3 ml/kg d-limonene did not decrease the blood pressure of rats, however. Authors also reported that isolated smooth muscles of the intestine, vas deferens, uterus, and peripheral vessel were constricted by d-limonene (Tsuji et al., 1974). Single high s.c. doses of d-limonene produced scratch behaviour and single high i.v. doses of d-limonene produced stretch behaviour in mice and rats (Tsuji et al., 1975a).

Table App. 1-1: Summary of acute toxicity studies published on d-limonene

Species	Sex	Route	LD ₅₀	Purity	GLP	reference
Mouse	M&F	Oral in juice, 7d	6.3 ml/kg (M) 8.1 ml/kg (F)	NS	N	Tsuji et al., 1974
Mouse	M&F	I.P. in juice, 3 d	3.7 ml/kg (M) 3.6 ml/kg (F)	NS	N	Tsuji et al., 1974
Mouse	M&F	I.P. in juice, 10 d	0.7 ml/kg (M) 0.6 ml/kg (F)	NS	N	Tsuji et al., 1974
Mouse	M&F	SC, in juice, 7d	>25.6 ml/kg (M) >25.6 ml/kg (F)	NS	N	Tsuji et al., 1974
Mouse	M&F	Oral	5600 mg/kg (M) 6600 mg/kg (F)	NS	N	Tsuji et al., 1975a
Mouse	M&F	Ip	1300 mg/kg (M) 1300 mg/kg (F)	NS	N	Tsuji et al., 1975a
Mouse	M&F	SC	>41500 mg/kg (M) >41500 mg/kg (F)	NS	N	Tsuji et al., 1975a
Rat	M&F	Oral	4400 mg/kg (M)	NS	N	Tsuji et al., 1975a

SPEARMINT OIL
Annex B.6: Toxicology and Metabolism

Species	Sex	Route	LD ₅₀	Purity	GLP	reference
			5100 mg/kg (F)			
Rat	M&F	Ip	3600 mg/kg (M) 4500 mg/kg (F)	NS	N	Tsuji et al., 1975a
Rat	M&F	SC	>20200 mg/kg (M) >20200 mg/kg (F)	NS	N	Tsuji et al., 1975a

Ip = Intraperitoneal. SC = subcutaneous NS : Not stated. N : No (due to the year of the studies)

Short term toxicity studies performed on limonene

Oral toxicity of limonene

Oral limonene toxicity studies in the rat

"16-dy" study

Authors: NTP (1990), National Toxicology Program Technical report on the toxicology and carcinogenesis studies of d-limonene (CAS No 5989-27-5) in F344/N rats and B6C3F1 mice (Gavage studies), NTP TR 347, NIH Publication No 90-2802.

Guideline: Not stated

GLP: Not stated

Material and methods:

Groups of 5 F344/N rats of each sex were given daily gavage doses of 0, 413, 825, 1650, 3300 or 6600 mg d-limonene/kg bw in 10 ml/kg bw corn oil 5 days/week over a 16 day period (12 total doses).

Findings: All rats receiving 6600 mg d-limonene/kg bw/day as well as 5/5 male and 3/5 female rats that were exposed to 3300 mg/bw/day died. The final mean body weight of male rats that received 1650 mg/kg bw/day was 10% lower than vehicle controls.

Conclusion: The authors reported that no clinical signs were seen in rats receiving 1650 mg d-limonene/kg bw/day or lower, and that no compound-related histopathologic effects were seen in any rats (NTP, 1990).
NOAEL = 825 mg/kg bw/day

"13-week" study

The following study is published in literature (Karnerva R.L., Ridder G.M., Lefever F.R., Alden C.L., 1987a) and was performed to compare short-term renal effects due to oral administration of decalin or d-limonene in young adult male Fischer-344 rats

Authors: Karnerva R.L., Ridder G.M., Lefever F.R., Alden C.L. (1987a), Comparison of short-term renal effects due to oral administration of decalin or d-limonene in young adult male Fischer-344 rats
Food and Chemical Toxicology, 25, 345-353

Guideline: Not stated

GLP: Not stated

Material and methods:

Groups of 5 male Fischer-344 rats were given single daily gavage doses containing 75, 150 or 300 mg d-limonene/kg bw in corn oil (5 ml/kg bw) 5 days/week for 5 or 20 doses, primarily to follow the development of renal alterations.

Findings/conclusion: The authors reported that no signs of gross toxicity were observed nor any effect on weight gain or feed consumption over the course of this experiment. However, after only 5 days of exposure, liver and kidney weights showed a dose-related increase and there was a dose-related increase in the number and size of hyaline droplets and accumulation of $\alpha_2\mu$ -globulin in the proximal convoluted tubule epithelial cells of the kidneys.

In addition, after 20 doses, granular casts in the outer zone of the medulla and multiple cortical changes (classified as chronic nephrosis) were also seen in dose-related severity (Kanerva *et al.*, 1987a).

NOAEL < 75 mg/kg bw/day

“30-day” study

The following study is published in literature (Tsuji M., Fujisaki Y., Arikawa K., Masuda S., Kinoshita S., Okubo A., Noda K., Ide H., Iwanaga Y. 1975a) and was performed to compare short-term renal effects due to oral administration of decalin or d-limonene in young adult male Fischer-344 rats

Authors: Tsuji M., Fujisaki Y., Arikawa K., Masuda S., Kinoshita S., Okubo A., Noda K., Ide H., Iwanaga Y. (1975a). Studies on d-limonene, as gallstone solubilizer (II): Acute and subacute toxicities. *Oyo Yakuri (Pharmacometrics)*, 9, 387-401

Guideline: Not stated

GLP: Not stated

Material and methods:

In a subacute study, groups of 5 male and female rats were exposed daily to 0, 277, 554, 1385 or 2770 mg d-limonene/kg bw orally for 30 days.

Findings: A general decrease in food intake and a dose-related decrease in body weight were seen in groups of exposed males, but little or no effect on neither organ weights nor relative organ weights was observed.

No significant changes were seen in urinalysis, haematology or biochemical values. The following tissues were examined histopathologically: adrenals, duodenum, heart, kidneys, liver, lungs, lymph nodes, pancreas, pituitary, spleen, stomach, testes/ovaries, thymus and thyroids.

Conclusion: Authors reported that no significant changes were noted except that granular casts were seen in the kidneys of most exposed male rats (0/5, 3/5, 5/5, 5/5 or 4/5 animals exposed to 0, 277, 554, 1385 or 2770 mg/kg bw/day respectively) (Tsuji *et al.*, 1975a).

NOAEL < 277 mg/kg bw/day

“13 week” study

A separate study of the slides containing the renal sections of the male rats exposed to d-limonene in this 13-week study was published in the literature (Karneva R.L., Alden C.L. (1987). Review of kidney sections from a subchronic d-limonene oral dosing study conducted by the National Cancer Institute; Food and Chemical Toxicology, 25, 355-358) and is summarised here in the context of the 13-week study.

Authors: NTP (1990), National Toxicology Program Technical report on the toxicology and carcinogenesis studies of d-limonene (CAS No 5989-27-5) in F344/N rats and B6C3F1 mice (Gavage studies), NTP TR 347, NIH Publication No 90-2802.

Guideline: Not stated

GLP: Not stated

Material and methods: Groups of 10 F344/N rats of each sex were given daily gavage doses of 0, 150, 300, 600, 1200 or 2400 mg d-limonene/kg bw in 5 ml/kg bw corn oil 5 days/week for 13 weeks.

Findings/conclusion: Five male and nine female rats exposed to 2400 mg d-limonene/kg bw/day died during the course of the study. In all rats receiving 1200 or 2400 mg/kg bw/day, rough hair coats, lethargy and excessive lacrimation were observed. Authors reported that nephropathy was seen in all groups of male rats, with a dose-related increase in severity. The nephropathy was reported to be characterized by degeneration of epithelium in the convoluted tubules, granular casts within tubular lumens (primarily in the outer stripe of the outer medulla), and regeneration of the tubular epithelium. In all groups of male rats (including control animals), hyaline droplets were observed in the epithelium of proximal convoluted tubules in at least some animals. An attempt to determine by "blind" evaluation if the incidence of these droplets was dose-related was reported to have resulted in an equivocal conclusion.

The slides containing the renal sections made from male rats exposed to d-limonene in this 13 week study were reviewed. They reported that hyaline droplet accumulation within the cytoplasm of proximal convoluted tubule (PCT) epithelial cells was uniform among all groups, including controls. However, chronic nephrosis, although seen in all animals (including control animals), appeared with a dose-related increase in severity. The authors attributed the uniformity of hyaline droplet accumulation to the fact that the animals were not exposed to d-limonene for 4-5 days before necropsy and examination, giving the animals time to rid themselves of some of the droplets, although more extensive damage to the kidneys (chronic nephrosis) was not repaired (Kanerva & Alden 1987).

Chronic nephrosis consisted of cytoplasmic basophilia of the PCT epithelial cells, tubular hyperplasia or atrophy, fibrosis of Bowman's capsule and an interstitial fibrolymphocytic response. Atrophic tubules were characterized by thickened basement membranes and a decrease in tubular cross-sectional diameter due to a decrease in cell size, whereas tubules considered to be hyperplastic showed increases in cell size and number and an increase in cross-sectional diameter compared with the surrounding unaffected tubules.

In addition, occasional foci of PCT epithelial cell necrosis/degeneration were observed in kidneys from all d-limonene-treated animals but not in control animals. All treated animals, but not controls, also showed a dose-related increase in the number of granular casts found in the outer stripe of the medulla. Granular casts were markedly dilated and had attenuated or non-existent epithelium. In addition, animals exposed to the highest dose level (2400 mg/kg bw/day) were reported to show multifocal hyaline casts and tubular dilation in the cortex and medulla (NTP, 1990).

NOAEL < 150 mg/kg bw/day

Oral limonene toxicity studies in the mouse

"16-day" study

Authors: NTP (1990), National Toxicology Program Technical report on the toxicology and carcinogenesis studies of d-limonene (CAS No 5989-27-5) in F344/N rats and B6C3F1 mice (Gavage studies), NTP TR 347, NIH Publication No 90-2802.

Guideline: Not stated

GLP: Not stated

Material and methods:

Groups of 5 B₆C₃F₁ male and female mice were given daily gavage doses of 0, 413, 825, 1650, 3300 or 6600 mg d-limonene/kg bw in 10 ml/kg bw corn oil 5 days/week over a 16 day period (12 total dosing).

Findings: All mice (5/5) exposed to 6600 mg/kg bw/day, 4/5 males and 5/5 females exposed to 3300 mg/kg bw/day, and 1/5 males and 1/5 females exposed to 1650 mg/kg bw/day died

Conclusion: before the end of the study. The one female that died at the latter dose level was reported to have been killed by a gavage error. Authors concluded that no compound-related clinical signs were observed in mice that received 1650 mg d-limonene/kg bw and lived to the end of the study, and that no compound-related histopathologic effects were seen (NTP, 1990). NOAEL = 1650 mg/kg bw/day

"13 week" study

Authors: NTP (1990), National Toxicology Program technical report on the toxicology and carcinogenesis studies of d-limonene (CAS No 5989-27-5) in F344/N rats and B6C3F1 mice (Gavage studies), NTP TR 347, NIH Publication No 90-2802.

Guideline: Not stated

GLP: Not stated

Material and methods:

Groups of 10 B₆C₃F₁ mice of each sex were given daily gavage exposures of 0, 125, 250, 500, 1000 or 2000 mg d-limonene/kg bw in 10 ml/kg bw corn oil 5 days/week for 13 weeks.

Findings: One male exposed to 2000 mg/kg bw/day, two females exposed to 2000 mg/kg bw/day, and one female exposed to 500 mg/kg bw/day, died during the course of the study. In addition, one female exposed to 125 mg/kg bw/day and five males (one each from the groups exposed to 250 and 1000 mg/kg bw/day, and three from the group exposed to 500 mg/kg bw/day) died prior to the end of the study, but their deaths were attributed by the authors to accidents related to the gavage procedure used. At the two highest dose levels, mice were seen with rough hair coats and decreased activity. An alveolar cell adenoma was observed in the lung of one female mouse that had received 2000 mg d-limonene/kg bw/day (NTP, 1990). NOAEL = 250 mg/kg bw/day

Oral toxicity studies in the dog

All these studies were summarized by WHO (Limonene-Food additives Series 30) as follows:

Groups of 3 male or female Japanese beagle dogs were exposed orally to 0, 0.4, 1.2 or 3.6 ml d-limonene/kg bw daily for 6 months. Dose-related occurrences of frequent vomiting and nausea were seen in some animals. At doses of at least 1.2 ml/kg bw/day for females and 3.6 ml/kg bw/day for males, a decrease in body weight gain was noted compared to controls, but little or no effect on food intake, organ weights or relative organ weights was reported. No significant changes were seen in urine analysis, haematology nor biochemical values, except that in animals at the 3.6 ml/kg bw/day level, total cholesterol and blood sugar were lowered. The following tissues were examined histopathologically: adrenals, bile duct, duodenum, heart, kidneys, liver, lungs, pancreas, pituitary, rectum, spleen, stomach, testes/ovaries, thymus and thyroids. No significant changes were noted except that granular casts were seen in the kidneys of all male dogs exposed to 3.6 ml/kg bw/day and all females exposed to 0.4 ml/kg bw/day or more (1/3, 1/3, 2/3 or 3/3 for males; 1/3, 2/2, 3/3 or 3/3 for females exposed to 0, 0.4, 1.2 or 3.6 ml/kg bw, respectively) (Tsuiji *et al.*, 1975b).

In a more recent study, groups of five male and female beagle dogs were gavaged twice daily for six months with 0, 0.12, or 1.2 ml/kg bw/day (0, 10, or 1000 mg/kg bw/day) d-limonene. The highest dose was reported to

be near the maximum tolerated dose for emesis. The authors stated that food consumption and body weight were not affected by treatment. Although there was a positive dose-related trend for absolute and relative female kidney weights and for relative male kidney weight, authors reported that there were no histo-pathological findings in the kidneys that could be associated with the organ weight changes. In addition, there was no hyaline droplet accumulation nor other indication of nephropathy such as those associated with d-limonene consumption in male rats (Webb *et al.*, 1990).

Genotoxicity studies performed on limonene

Table. App.1-2: Genotoxicity data on d-limonene

Test system	Test object	Concentration of d-limonene	Results	Reference
Ames test (+ & - S9)	S. typhimurium TA98, TA100, TA1535, TA1537	0.03-3 $\mu\text{mol/plate}$	Negative	Florin et al., 1980
Yeast (- S9)	S. cerevisiae MP1 strain cells	0.1-100 $\text{mM}/5 \times 10^7$	(1)	Fahrig, 1984
Mammalian (- S9)	Mouse embryo system	0-215 mg/kg	(2)	Fahrig, 1984
Ames test (+ & - S9)	S. typhimurium TA1535? TA100, TA1537, TA1538, TA98	0-20 $\mu\text{M/plate}$	Negative	Watabe et al., 1980
Ames test (+ & - S9)	S. Typhimurium, TA100, TA1535, TA1537, TA98	0-3333 $\mu\text{g/plate}$	Negative	Haworth et al., 1983
Ames test (+ & - S9)	S. Typhimurium, TA 100, TA1535, TA1537, TA98	0-3333 $\mu\text{g/plate}$	Negative	NTP, 1990
Mammalian Cells (+ & - S9)	Mouse L5178Y, cells in vitro		Negative	NTP, 1990
Sister chromatid exchange (+ & - S9)	Chinese hamster ovary cells in vitro	0-162 $\mu\text{g/ml}$	Negative	NTP, 1990
Chromosomal aberrations (+ & - S9)	Chinese hamster ovary cells, in vitro	0-500 $\mu\text{g/ml}$ (3) 0-100 $\mu\text{g/ml}$ (4)	Negative Negative	NTP, 1990
Ames test (+ & - S9)	S. Typhimurium, TA1535, TA1537, TA1538, TA98, TA100	150 mg/plate	Negative	Heck et al., 1989
Mammalian Cells (+ & - S9)	Mouse L5178Y cells in vitro	100 $\mu\text{g/ml}$	Negative	Heck et al., 1985

(1) Author reported that results indicated that d-limonene was a co-recombinant and an anti-mutagen.

(2) Author reported that results indicated that d-limonene is inactive when given alone, but reduces the mutagenic effect of ethylnitrosourea when the two are co-administered.

(3) Activated: addition of S9 from the livers of Aroclor 1254-induced male Sprague Dawley rats.

(4) Not activated

Carcinogenicity studies performed on limonene

All these studies were summarized by WHO (Limonene-Food additives Series 30) as follows:

Carcinogenicity studies in the rat

Groups of fifty 7-8 week old male F344/N rats were given daily gavage doses of 0, 75, or 150 mg d-limonene/kg bw and groups of 50 7-8 week old female F344/N rats were given 0, 300, or 600 mg d-limonene/kg bw in 5 ml/kg bw corn oil 5 days/week for 103 weeks.

Rats were observed twice a day; they were weighed weekly for the first 12 weeks and monthly thereafter. Clinical signs were recorded at least monthly. Moribund animals were killed; necropsies were performed on all available animals. The tissues of all rats that died during the study, animals with grossly visible lesions, and all animals in the control and high dose groups were examined for histopathology.

No compound-related clinical signs were reported. The survival of the female animals exposed to 600 mg/kg bw was lower than that of control animals after week 39, while the survival of male rats exposed. Mean body weights of male rats exposed to 150 mg/kg bw were generally 4%-7% lower than controls from week 28. Cataracts were observed at increased incidence in male rats exposed to 150 and female rats exposed to 300 or 600 mg d-limonene/kg bw/day, and retinal degeneration was seen in all dosed animals. The authors pointed out, however, that the rats of each sex that were exposed to their respective highest-dose levels were housed in the top rows of the cage racks, the animals exposed to the low-dose levels were below them, and the controls were housed in the lowest rows for all but the last 10 weeks of the study. Authors conclude that these effects were due to variations in the proximity of animals in different dose groups to the light source.

Subcutaneous tissue fibromas in male rats occurred with a negative dose-response trend (controls: 8/50; 75 mg/kg bw/day: 2/50; 150 mg/kg bw/day: 3/50). However, the combined incidence of fibromas and fibrosarcomas was not significantly different when dosed males were compared to controls (controls: 8/50; 75 mg/kg bw/day: 4/50; 150 mg/kg bw/day: 3/50).

Combined squamous cell papillomas or carcinomas in the skin of male rats showed a positive dose-response trend (controls: 0/50; 75 mg/kg bw/day: 0/50; 150 mg/kg bw/day: 3/50). However, the incidence of these neoplasms in dosed male rats was within the range of incidences for NTP historical control rats, and did not differ significantly from the incidence in control animals. The authors concluded that the positive dose-response trend was not related to d-limonene exposure.

Mononuclear cell leukaemia occurred in male rats with a positive dose-response trend by the incidental tumour test (controls: 10/50; 75 mg/kg bw/day: 10/50; 150 mg/kg bw/day: 19/50). The incidences in dosed male rats were not significantly different from the incidences in controls, however. The authors concluded that the positive dose-response trend was not related to d-limonene administration. There was a significant positive dose-response trend in the incidence of interstitial cell tumours in the testes of male rats, and the incidences of these tumours in dosed groups were significantly higher than the incidences in control animals (control: 37/50; 75 mg/kg bw/day: 47/49; 150 mg/kg bw/day: 48/50). The authors argued, however, that since historically this type of

tumour occurs in almost all aged F344 rats, this result should not be considered to be related to d-limonene exposure, but rather was an artefact caused by the low survival of the control animals in this experiment (see above).

The incidence of uterine endometrial stromal polyps in females exposed to 300 mg/kg bw/day was increased when compared to controls. However, the incidence in the control animals was well below the NTP historical incidence of this tumour. For this reason, and because there was no positive dose-response trend, the authors argued that this was not a compound-related effect.

d-Limonene exposure of male rats was associated with dose-related increases in the incidences of mineralization and epithelial hyperplasia in the kidneys (mineralization: controls - 7/50; 75 mg/kg bw/day - 43/50; 150 mg/kg bw/day - 48/50) (epithelial hyperplasia: controls - 0/50; 75 mg/kg bw/day - 35/50; 150 mg/kg bw/day - 43/50). The lesions consisted of linear deposits of mineral in the medulla (renal papilla) and focal hyperplasia in the transitional epithelium overlying the papilla. Hyperplasia was frequently located near the fornices of the renal pelvis and was occasionally bilateral. The severity of nephropathy, which occurs spontaneously in aged male rats, was also positively dose-related in this experiment (severity of nephropathy, rated on a scale from 0 [not present] to 4 [marked]: control mean - 1.5; 75 mg/kg bw/day mean - 1.8; 150 mg/kg bw/day mean - 2.2). This nephropathy was characterized by degeneration and atrophy of the tubular epithelium, dilation of tubules with formation of hyaline droplets and granular casts, regeneration of tubular epithelium, glomerulosclerosis, and interstitial inflammation and fibrosis.

Kidney tubular cell hyperplasia and neoplasms were also increased in dosed male rats (hyperplasia: controls - 0/50; 75 mg/kg bw/day - 4/50; 150 mg/kg bw/day - 7/50) (adenomas: controls - 0/50; 75 mg/kg bw/day - 4/50; 150 mg/kg bw/day - 8/50) (adenocarcinomas: controls - 0/50; 75 mg/kg bw/day - 4/50; 150 mg/kg bw/day - 3/50) (combined adenomas and carcinomas: controls - 0/50; 75 mg/kg bw/day - 8/50; 150 mg/kg bw/day - 11/50). Incidences of tubular cell adenomas and the combined incidences of adenomas and adeno-carcinomas in male rats showed significant positive dose-response trends. High-dose males had a significantly higher incidence than control males of both categories of tumours, while low-dose males had a significantly higher incidence of the combined category than control males. These neoplasms are historically rare and none were seen in control males or in any group of females.

The authors stated that kidney tubular cell hyperplasia, adenomas, and adenocarcinomas were part of a morphologic spectrum. Proliferative lesions diagnosed as hyperplasia generally consisted of one to three adjacent cross-sections of enlarged tubules with stratification of the epithelium. In some lesions, the epithelial cells appeared to completely fill the lumen. The tubular cell neoplasms varied in diameter from less than 1 mm to 15 mm. They exhibited varied patterns of growth and were solid, cystic, or papillary. The solid and cystic neoplasms showed little evidence of tubular structures and the cells were arranged in solid sheets or small solid nests separated by a delicate vascular stroma. Some neoplasms were reported to consist of layers of epithelium lining a fibrous connective tissue stroma and were arranged in complex branching papillary formations. The

neoplasms were classified as adenocarcinomas primarily if they exhibited cellular pleomorphism and anaplasia, or if they were larger than 10 mm (NTP, 1990).

Carcinogenicity studies in the mouse

To assess the pulmonary tumour carcinogenicity of *d*-limonene, groups of 15 male or female A/He mice were injected i.p. with *d*-limonene 3 times weekly for 8 weeks (4.8 or 24 g/kg bw total dose) and then necropsied 24 weeks after the first injection. No significant induction of lung tumours was seen (Stoner *et al.*, 1973).

Groups of 50 8-9 week old male B₆C₃F₁ mice were given daily gavage doses of 0, 250, or 500 mg *d*-limonene/kg bw and groups of 50 8-9 week old female B₆C₃F₁ mice were given 0, 500, or 1000 mg *d*-limonene/kg bw in 10 ml/kg bw corn oil 5 days/week for 103 weeks. Mice were observed twice a day and weighed weekly for the first 12 weeks, after which they were weighed monthly. Clinical signs were recorded at least monthly. Moribund animals were killed and a necropsy was performed on all available animals. The tissues of all mice that died during the study and animals with grossly visible lesions, as well as all animals in the control and high dose groups, were examined for histopathology.

The authors reported that no clinical signs were associated with administration of the test chemical. The survival of male mice exposed to 250 mg *d*-limonene/kg bw/day was lower than that of controls at the end of the study (controls: 33/50; 250 mg/kg bw/day: 24/50; 500 mg/kg bw/day: 38/50). No other differences in survival were seen among exposed groups of either sex. Mean body weights of exposed and control male mice were generally comparable. Weights of female mice in the 1000 mg *d*-limonene/kg bw/day group, however, were 5-15% lower than control animals after week 28.

Multinucleated liver hepatocytes and cells with cytomegaly occurred at increased incidence in male mice exposed to 500 mg *d*-limonene/kg bw/day (multinucleated hepatocytes: controls - 8/49; 250 mg/kg bw/day - 4/36 [incomplete sampling]; 500 mg/kg bw/day - 32/50) (hepatocytes with cytomegaly: controls - 23/49; 250 mg/kg bw/day - 11/36 [incomplete sampling]; 500 mg/kg bw/day - 38/50). The combined incidence of hepatocellular adenomas and carcinomas, however, did not differ significantly from their incidence in control mice (controls: 22/49; 250 mg/kg bw/day: 14/36 [incomplete sampling]; 500 mg/kg bw/day: 15/50). No significant differences were observed in female mice for any of these endpoints.

The incidence of adenomas or the combined incidence of adenomas and carcinomas in the anterior pituitary gland of female mice exposed to 1000 mg *d*-limonene/kg bw/day were significantly lower than controls. However, the incidence of hyperplasia did not differ significantly from controls (hyperplasia: controls - 16/49; 500 mg/kg bw/day - 0/8 [incomplete sampling]; 1000 mg/kg bw/day - 17/48) (adenomas: controls - 12/49; 500 mg/kg bw/day - 5/8 [incomplete sampling]; 1000 mg/kg bw/day - 1/48) (combined adenoma and carcinoma: controls - 12/49; 500 mg/kg bw/day - 5/8 [incomplete sampling]; 1000 mg/kg - 2/48) (NTP, 1990).

Cocarcinogenicity/tumour promotion studies

All these studies were summarized by WHO (Limonene-Food additives Series 30) as follows:

Two publications have reported the results of experiments in which 0.25 ml oil of sweet orange, composed of > 90% d-limonene, was applied to the skin of 10 male and 10 female "101" mice weekly for 42 weeks, starting 3 weeks after an application of 300 µg 7,12-dimethyl-1,2-benzanthracene (DMBA) in 0.2 ml acetone. While no effects in the area of the treated skin were seen in mice exposed to d-limonene or to DMBA alone, mice exposed to DMBA followed by exposure to d-limonene began to develop papillomas during the twelfth week of d-limonene exposure. After 33 weeks of treatment, 13/18 treated mice showed a total of 39 papillomas. No malignant tumours were seen in the exposed area, however.

In a second study, mice were exposed to d-limonene following exposure to: 1) 4 skin applications of 60 mg urethane in 0.3 ml acetone at 3 day intervals, or 2) 4 i.p. injections of 16 mg urethane in 0.1 ml distilled water at 3 day intervals. Papillomas appeared on 3/13 mice in group 1 and 2/13 mice in group 2 by the fourteenth week of exposure.

In a final experiment, the terpene fraction of oil of sweet orange (containing d-limonene) was isolated and tested with DMBA as described for the first experiment. Papillomas in mice exposed to both DMBA and the terpene fraction of oil of sweet orange began to appear in the eleventh week of treatment and were visible in 8/15 mice by the thirty-third week of treatment. Although pure d-limonene was not tested in these experiments, the authors concluded that the promoting effects seen were probably caused by this chemical since it was the primary constituent of the test agent (Roe, 1959, Roe & Peirce, 1960).

A group of 23 male and female albino mice were given single doses of 50 µg benzo(a)pyrene (BAP) by stomach tube followed by 40 weekly doses of 0.05 ml d-limonene; seventeen male and female mice received BAP only, 15 male and female mice received d-limonene only and 18 male and female mice served as untreated controls. While no tumours of the forestomach epithelium were seen in control animals, 2 animals treated with BAP alone (12%), 2 animals treated with d-limonene alone (13%), and 5 animals treated with both BAP and d-limonene (22%), developed 0, 2, 3, and 8 tumours, respectively, although none was judged to be a carcinoma. The authors concluded that although d-limonene was weakly carcinogenic for mouse forestomach epithelium it did not increase the tumour yield caused by pre-treatment with BAP and, therefore, was not a promoter (Field & Roe, 1965).

Cells of embryos taken from F344 rats were infected with Rauscher murine leukaemia virus and then treated with 0.05 µg/ml 3-methylcholanthrene (3-MCA) followed by 15 µg/ml d-limonene. Following treatment with 3-MCA and d-limonene, colony formation in semisolid medium was observed and was considered to denote cell transformation. Authors reported that d-limonene alone failed to transform cells and 3-MCA alone was only effective at concentrations exceeding 0.2 µg/ml (Roe, 1959; Roe & Peirce, 1960; Traul *et al.*, 1981)).

Elegbede and co-workers attempted to repeat the 1960 study of Roe and Peirce (see above) using purified *d*-limonene as well as oil of sweet orange. The skin of groups of 24 female CD-1 mice were treated once with 51.2 µg DMBA in 0.2 ml acetone followed 14 days later by twice weekly applications of 0.1 ml *d*-limonene or oil of sweet orange mixed with 0.1 ml acetone. Other groups of mice were similarly initiated with DMBA, but beginning 7 days later were given a diet containing 1% *d*-limonene or oil of sweet orange. In all cases, administration of *d*-limonene or oil of sweet orange continued for 40 weeks after DMBA treatment. Topical (but not ingested) *d*-limonene was found to slightly increase the number of tumours induced by DMBA alone, the increase becoming significant only after 34 weeks of treatment. When oil of sweet orange (95% *d*-limonene content) was applied to the skin, authors reported that a promoting effect was seen after 7 weeks of treatment; promotion was not reported, however, when oil of sweet orange was incorporated into the diet. The authors concluded that a component of orange oil other than *d*-limonene was responsible for the promoting activity observed and that the very small promoting activity seen with *d*-limonene may be due to its contamination by this unidentified component (Elegbede *et al.*, 1986b).

Anticarcinogenicity studies

All these studies were summarized by WHO (Limonene-Food additives Series 30) as follows:

A group of 50 male C57Bl/6J mice were injected s.c. with 25 µg dibenzpyrene (DBP) followed 24 h later by an injection of 0.15 ml *d*-limonene. After 34 weeks of observation, the authors reported that *d*-limonene significantly delayed the appearance of lung adenomas induced by DBP and reduced the total number of tumours seen. At 30 weeks post-DBP exposure without subsequent exposure to *d*-limonene, lung adenomas were seen in approximately 70% of the animals; with exposure to *d*-limonene, tumours were seen in approximately 30% of the animals.

In a second experiment reported in the same paper, a group of 50 female A/J mice were injected s.c. with DBP followed by 16 weekly injections into the tail vein of 1% v/v suspensions of 1 mg of *d*-limonene. The incidence of lung adenomas was reduced from 75% of animals exposed to DBP alone to 40% of animals exposed to DBP and injected with *d*-limonene. Control incidence of lung adenomas in this experiment was 27%. Treatment of mice with *d*-limonene alone reduced this background incidence to approximately 7% (Homburger *et al.*, 1971).

Simultaneously, 5 µg of BAP and 10 mg *d*-limonene were applied to the skin of groups of 50 female ICR/Ha Swiss mice three times a week for 440 days. *d*-Limonene was found to partially inhibit BAP carcinogenicity (Van Duuren & Goldschmidt, 1976).

Female Sprague-Dawley rats were fed diets containing 0, 1000 or 10 000 ppm *d*-limonene for 1 wk, at which time they were given a single gavage dose of 65 mg DMBA/kg bw. The *d*-limonene-containing diet was then continued for 27 weeks. Authors reported that *d*-limonene delayed the appearance of DMBA-induced mammary tumours in a dose-related manner, reduced the incidence of tumours, and caused increased regression of tumours that did appear (Elegbede *et al.*, 1984a,b).

Female (W/Fu x F344)F₂ rats were fed a diet containing 10% d-limonene for 80 days beginning after the first appearance of tumours induced by intubation of rats with 130 mg DMBA/kg bw.

Significant tumour regressive action by d-limonene was observed, and the development of subsequent tumours was also reduced significantly (Elegbede *et al.*, 1986a).

Following initiation with DMBA (single gavage dose of 65 mg/kg in sesame oil), groups of female Sprague-Dawley rats were (1) fed a basal cereal diet one week before and 24 weeks following initiation with DMBA (controls), (2) fed a diet containing 5% d-limonene one week before and one week after treatment with DMBA, then fed the control diet for 24 weeks (initiation group), or (3) fed the control diet for one week before and one week following DMBA initiation, then fed the 5% d-limonene diet for 24 weeks (promotion/progression group). d-Limonene was reported to be effective in reducing the average number of rat mammary carcinomas in DMBA-initiated rats when fed during the initiation phase or during the initiation/progression phase of carcinogenesis. Time to appearance of the first tumour was extended only when d-limonene was fed during the initiation phase, however. Authors concluded that these effects could not be attributed to changes in mammary-relevant endocrine functions (serum levels of prolactin and duration of estrus cycle) (Elson *et al.*, 1988).

Female Wistar-Furth rats were (1) placed on diets containing 5% d-limonene or 5% orange oil (source of d-limonene) for 2 weeks prior to initiation with nitrosomethylurea (NMU; single i.v. dose of 50 mg/kg bw), then continued on these diets for 23 weeks; (2) fed diets containing 5% d-limonene for two weeks before and one week following initiation with NMU, then fed a basal diet for 22 weeks (initiation group); or (3) fed basal diets prior to and for 1 week following initiation with NMU, then fed diets containing 5% d-limonene for 22 weeks (promotion/progression group). Both orange oil and d-limonene decreased mammary tumour incidence (controls: 80%; d-limonene: 45%; orange oil: 47%) and the average number of mammary tumours per rat (controls: 1.8; d-limonene: 0.4; orange oil: 0.8) when present in the diet for two weeks before and 23 weeks after NMU administration. d-Limonene decreased tumour incidence and decreased the numbers of tumours per rat (by approximately 50%) when fed during the promotion/progression experiment but not the during the initiation experiment (Maltzman *et al.*, 1989).

Studies on the mechanism of d-limonene carcinogenicity

All these studies were summarized by WHO (Limonene-Food additives Series 30) as follows:

This section discusses (1) spontaneously occurring nephropathy in mature male rats and (2) the current proposed mechanism of carcinogenicity of d-limonene and other chemicals/mixtures that exacerbate this condition and lead to alpha_{2u}-globulin-associated male rat nephropathy.

(1) Mature male rat nephropathy

Normal male rats at least 30 days old exhibited marked increased protein excretion in their urine not seen in younger males or in females of any age. This proteinuria reached a maximum level of approximately 2.5-3 times that seen in females at 90 days of age.

Microscopically, the authors also observed intracellular droplets in the epithelial cells of the upper two-thirds of the proximal convoluted tubules of kidneys of male rats at least 60 days old. The occurrence of these hyaline droplets consisted of a few groups of small droplets in occasional cells in 60 day old animals. The number of cells having droplets and the number and size of droplets per cell increased with age; from 90 days of age most proximal tubule cells contained them. In contrast, at 120 and 180 days of age, only a small number of females showed occasional droplets in a few cells of the proximal tubules.

These authors further demonstrated the sex-relatedness of this phenomenon by castrating male rats at 30 days of age and injecting female rats with 5 mg testosterone every other day from 50 days of age. When examined at 120 days of age, the castrated male rats showed proteinuria at a level equal to control females and hyaline droplets were only seen in 4/20 animals. Testosterone-treated females, on the other hand, had proteinuria at a level approximately midway between control males and females. No hyaline droplets were seen in the proximal tubule cells of these females, however, showing that the addition of testosterone alone was insufficient to cause them to appear (Logothetopoulos & Weinbren, 1955).

Numerous authors have since reported detailed descriptions of spontaneously occurring nephropathy in mature male rats which is a consequence of this hyaline droplet accumulation. The sequence of events appears to be that: (1) protein accumulates in the lysosomes in the cytoplasm of epithelial cells of the P₂ segment of the proximal convoluted tubules in the kidney cortex; (2) the accumulated material becomes so abundant that it crystallizes, forming the microscopically visible hyaline droplets; (3) the continued build-up of material eventually leads to the death of epithelial cells with a concomitant thinning of the epithelial layer (although some regeneration is usually seen); and (4) the dead cell debris becomes lodged in the outer strip of the outer medulla where the tubules narrow, forming granular casts and causing tubule dilation with pressure necrosis of the cells of the tubule walls.

There is also an increase in the relative weight of the kidneys accompanying this phenomenon.

A steadily increasing number of agents have been identified that appear to exacerbate this hyaline droplet formation and its consequences in mature male rats. With some, noticeable changes occur within days of exposure. These agents include complex mixtures of hydrocarbons such as mineral spirits (Carpenter *et al.*, 1975a; Phillips & Cockrell, 1984), "60" solvent (Carpenter *et al.*, 1975b), "high naphthenic solvent" (Carpenter *et al.*, 1977), the petroleum-derived or shale-derived jet fuel JP-5 (MacEwen & Vernot, 1978a; Parker *et al.*, 1981; Bruner, 1984; MacNaughton & Uddin, 1984), petroleum-derived and shale-derived diesel fuel marine (DFM), the jet fuels JP-4, JP-TS and JP-7, and the missile propellants JP-10 and RJ-5 (Bruner, 1984; MacNaughton & Uddin, 1984), C/10-C/11 isoparaffin (Phillips & Egan, 1984; Phillips & Cockrell, 1984), five

petroleum naphtha mixtures (Halder *et al.*, 1984) and unleaded gasoline (MacFarland *et al.*, 1984; Busey & Cockrell, 1984; Halder *et al.*, 1984; Kitchen, 1984; Garg *et al.*, 1988a,b, 1989; Short & Swenberg, 1988; Short *et al.*, 1989a).

Also included on the list are the individual chemicals decahydro-naphthalene (decalin, MacEwen & Vernot, 1978b; Gaworski *et al.*, 1980, 1981; Bruner, 1984; Alden *et al.*, 1984; Olson *et al.*, 1986; Stone *et al.*, 1987a,b; Kanerva *et al.*, 1987b,c; Read, 1988), pentachloroethane (NTP, 1983; Goldsworthy *et al.*, 1988), 1,4-dichlorobenzene (NTP, 1987a; Charbonneau *et al.*, 1989), perchloroethylene (Goldsworthy *et al.*, 1988), dimethyl methylphosphonate (NTP, 1987b), 2,2,4-trimethylpentane (Stonard *et al.*, 1986; Short *et al.*, 1987; Charbonneau *et al.*, 1987; Lock *et al.*, 1987; Loury *et al.*, 1987; Short *et al.*, 1989a), the chemically unrelated agents 3-methylamino-1-(3-trifluoromethyl-phenyl)-2-pyrazoline, *cis/trans*-2-(4'-*t*-butylcyclohexyl)-3-hydroxy-1,4-naphthoquinone and 2,3,5,6-tetrahydro-6-phenylimidazo-(2,1-b) thiazole (Read *et al.*, 1988) and *d*-limonene (Kanerva *et al.*, 1987a; NTP, 1990).

There is no obvious common structure among the specifically identified chemicals that cause an increased accumulation of hyaline droplets in mature male rat kidneys. And there are examples of agents that do have similar mixtures/structures to those listed above that do not cause this phenomenon. These include "70" solvent (Carpenter *et al.*, 1975c), two other petroleum naphtha mixtures (Halder *et al.*, 1984), 1,2-dichlorobenzene (Charbonneau *et al.*, 1989) and an analog of *d*-limonene, 4-vinylcyclohexene (NTP, 1986).

Recently, however, Miller *et al.* (1989) reported that they have identified correlations between structure and binding affinity to $\alpha_{2\mu}$ -globulin, but no specific details were given in their published report.

While exposure to unleaded gasoline for 72 h has been shown to cause effects that are reversible (Garg *et al.*, 1988a), with chronic exposure to several of these mixtures/chemicals the kidney nephrosis has been reported to progress to hyperplasia, adenoma and even adenocarcinoma (MacFarland *et al.*, 1984; Kitchen, 1984; MacNaughton & Uddin, 1984; NTP, 1987a,b,c; NTP, 1990).

(2) Mechanism for $\alpha_{2\mu}$ -globulin-associated male rat nephropathy

The mechanism proposed for $\alpha_{2\mu}$ -globulin-associated male rat nephrosis has recently been thoroughly reviewed by the US Environmental Protection Agency (US EPA, 1991). Their review incorporated the results of many studies, including Charbonneau & Swenberg (1988), Swenberg *et al.* (1989), Flamm & Lehman-McKeeman (1991), Lehman-McKeeman *et al.* (1989), Lehman-McKeeman *et al.* (1990a,b), Short *et al.* (1987), Short *et al.* (1989a,b), Dietrich & Swenberg (1991), Murty *et al.* (1988), Ridder *et al.* (1990), and Webb *et al.* (1989).

The review concluded that:

"Of the eight model substances tested in chronic animal bioassays (decalin, dimethyl methyl phosphonate, JP-4 jet fuel, JP-5 shale-derived jet fuel, *d*-limonene, methyl isobutyl ketone, pentachloroethane, and unleaded

gasoline), all invoked a specific type of protein droplet nephropathy in male rats and also produced renal tumours in male rats but not in other species tested. It has been proposed that such renal tumours are the end product in the following sequence of functional changes in the epithelial cells of proximal tubules:

- Excessive accumulation of hyaline droplets in proximal tubules, representing lysosomal overload, cell loss, and regenerative cellular proliferation.
- Cell debris in the form of granular casts accumulates at the 'corticomedullary' junction with associated dilation of the affected tubule segment and more distally, mineralization of tubules within the renal medulla.
- Single-cell necrosis accompanied by compensatory cell proliferation and exacerbation of the chronic progressive nephropathy characteristically found in aging rats occurs.
- Renal tubule hyperplasia and neoplasia develop subsequently.

According to this hypothesis, the increased proliferative response caused by the chemically induced cytotoxicity results in clonal expansion of spontaneously initiated renal tubule cells and increased incidence of renal tumour formation (Trump *et al.*, 1984a; Alden, 1989; Swenberg *et al.*, 1989). This line of reasoning leads supporters of the hypothesis to conclude that the acute and chronic renal effects induced in male rats by such chemicals will be unlikely to occur in any species not producing $\alpha_{2\mu}$ -globulin, or a very closely related protein, in the large quantities typically seen in the male rat (Alden, 1989; Borghoff *et al.*, 1990; Green *et al.*, 1990; Olson *et al.*, 1990; Flamm & Lehman-McKeeman, 1991; Swenberg, 1991)."

The following paragraphs summarize some of the data that support this hypothesis:

Contents and catabolism of hyaline droplets/ phagolysosomes

The composition of the hyaline droplet inclusions has been identified by Kanerva *et al.* (1987c) using two-dimensional gel electrophoresis and immunological binding as being four species of the low molecular weight protein $\alpha_{2\mu}$ -globulin. As expected, $\alpha_{2\mu}$ -globulin was found to be absent in immature male rats and female rats of all ages. It was present, however, in female rats that had been ovariectomized and repeatedly injected with testosterone. More recently, groups have confirmed that $\alpha_{2\mu}$ -globulin is the major constituent of the renal phagolysosomal inclusions in male rats exposed to unleaded gasoline (Garg *et al.*, 1988b), and perchloroethylene and penta-chloroethane (Goldsworthy *et al.*, 1988). Finally, Dietrich and Swenberg (1991) reported that male NCI-Black-Reiter rats, which do not produce $\alpha_{2\mu}$ -globulin, are not susceptible to nephropathy produced by 2,2,4-trimethylpentane, 1,4-dichloro-benzene, isophorone, PS-6 unleaded gasoline, or d-limonene.

A sex-specific protein has been identified in human urine (Bernard *et al.*, 1989), but the concentration (up to 108 $\mu\text{g/l}$) is about 10 000 times less than that of $\alpha_{2\mu}$ -globulin in male rats (about 40 mg/24 h) (Roy & Neuhaus, 1967). Therefore assuming that the protein in human urine shows the same binding characteristics as $\alpha_{2\mu}$ -globulin, it is reasonable to conclude that humans would be comparably less sensitive than the male rat.

Alpha_{2μ}-globulin is synthesized in the liver of rats of both sexes. This protein is filtered by the kidney glomeruli and reabsorbed by the epithelial cells of the proximal convoluted tubules by the formation of phagosomes at the luminal surface.

Normally, the phagosomes then combine with cellular lysosomes and the protein is degraded. In mature male rats, however, this catabolism is slowed in some way under hormonal influence leading to the intracellular accumulation of the protein which becomes manifested as hyaline droplets. The degradation is slowed even more by exposure to at least some of the agents listed in the previous section (Stonard *et al.*, 1986; Short *et al.*, 1987; Kanerva *et al.*, 1987c; Charbonneau *et al.*, 1987). A time- and dose-dependent progressive increase in the number and size, and alterations in the morphology of phagolysosomes in proximal convoluted tubule epithelial cells have been demonstrated with exposure to unleaded gasoline (Garg *et al.*, 1989). The ability of unleaded gasoline to induce these effects declines with the age of the male rat, in proportion to the declining production of alpha_{2μ}-globulin (Murty *et al.*, 1988).

Binding of chemicals to alpha_{2μ}-globulin

1,4-Dichlorobenzene as well as its major metabolite, 2,5-dichlorophenol, were both found to be reversibly bound (and 1,4-dichlorobenzene also covalently bound) to alpha_{2μ}-globulin in the kidneys of treated male rats (Charbonneau *et al.*, 1989).

Similarly, two groups have attempted to identify the specific binding between one of these agents (2,2,4-trimethylpentane) and alpha_{2μ}-globulin. Loury *et al.* (1987) reported that no covalent binding could be found, nor did there appear to be formation of a Schiff base. Lock *et al.* (1987), however, detected reversible binding of the metabolite 2,4,4-trimethyl-2-pentanol to alpha_{2μ}-globulin. This specific metabolite was found in liver cells of male rats only.

Likewise with decalin, an alcohol metabolite has been identified that is unique to male rats (Olson *et al.*, 1986). While both male and female rats synthesize *cis,cis*-2-decalol and *trans,cis*-2-decalol from *cis*-decalin in their livers, *cis,cis*-1-decalol was found only in males and *cis,trans*-1-decalol was produced in much higher concentrations than in females. In similar fashion, with *trans*-decalin as the substrate *trans,trans*-1-decalol was seen only in livers of male rats. When kidneys were examined, 2-decalone (*cis* or *trans*, depending on the original substrate) was found in the males while no decalin metabolites whatever were seen in female kidneys. Whether these unique male decalin metabolites react with alpha_{2μ}-globulin has not yet been tested.

More recently, Lehman-McKeeman *et al.* (1989) have reported that d-limonene and d-limonene-1,2-oxide, a major metabolite of d-limonene, were found reversibly bound to alpha_{2μ}-globulin (identified by amino acid sequencing) in the kidneys of treated male, but not female, rats.

Inhibition of $\alpha_2\mu$ -globulin catabolism

Charbonneau *et al.* (1988) reported that $\alpha_2\mu$ -globulin bound in kidney cytosol was not digested by purified proteases or proteinase.

Treating rats with leupeptin, an inhibitor of cathepsin B, a major lysosomal peptidase, mimics the ability of unleaded gasoline to cause the accumulation of $\alpha_2\mu$ -globulin in kidney phagolysosomes. Authors concluded that the mechanism of nephrotoxicity involves inhibition of renal phagolysosomal proteolysis (Olsen *et al.*, 1988).

Induction of cell proliferation

Short *et al.* (1989a) have shown that proliferation of epithelial cells in the P₂ proximal tubule segment in male rats is associated with exposure of the animals to either 2,2,4-trimethylpentane or unleaded gasoline. They noted that this proliferation closely paralleled the extent and severity of detectable $\alpha_2\mu$ -globulin in the same cells. The authors speculated that this proliferation leads to the nephrocarcinogenic effects caused by these agents. Proliferation was also noted by Charbonneau *et al.* (1989) in male rats treated with 1,4-dichlorobenzene. Short *et al.* (1989b) observed increased numbers of atypical cell foci and renal cell tumours in male, but not female, rats initiated with N-ethyl-N-hydroxyethylnitrosamine and subsequently exposed to unleaded gasoline or 2,2,4-trimethylpentane. The extent of the promotion was reported to be dose-related and to parallel the effects of these compounds on chronic cell proliferation.

Reproductive toxicity

Pregnant mice were given oral doses of 0, 591, or 2363 mg/kg bw d-limonene from days 7 through 12 of gestation. Authors reported significant decreases in body weight gain of dams in the high-dose group; fetuses of dams exposed to the high-dose showed increased incidences of lumbar rib, fused rib, delayed ossification, and decreased body weight gain relative to fetuses of control dams (Kodama *et al.*, 1977a).

Pregnant Japanese white rabbits were given oral doses of 0, 250, 500, or 1000 mg/kg bw d-limonene from day 6 to day 18 of gestation. Significant decreases in body weight gain in dams given 250 or 500 mg/kg bw/day d-limonene were observed, and survival in dams given 1000 mg/kg bw/day was significantly reduced (40% survival). From examination of the fetuses, authors concluded that d-limonene was not teratogenic in rabbits (Kodama *et al.*, 1977b).

d-Limonene was reported to increase the number of abnormal chick embryos to approximately 50% when a single dose (25 μ M/embryo) in olive oil was injected suprablastodermically (Abramovici & Rachmuth-Roizman, 1983).

Delayed neurotoxicity

No data available.

Further toxicological studies on limonene

Special limonene studies on immune response

All these studies were summarized by WHO (Limonene-Food additives Series 30) as follows:

Limonene has been identified as a primary skin irritant in humans (Pirila *et al.*, 1964; Klecak *et al.*, 1977), rabbits (research Institute for the Development of Fragrance Materials, Inc., 1984; Research Institute for Fragrance Materials Inc., 1985), guinea-pigs (Klecak *et al.*, 1977), and mice (Gad *et al.*, 1986).

d-Limonene was reported both to elicit (De Groot *et al.*, 1985) and not to elicit (Greif, 1967) allergic reactions in human skin and to elicit allergic reactions in mouse skin (Gad *et al.*, 1986).

d-Limonene was reported to eliminate human skin sensitization to several aldehydes when they were co-administered with d-limonene (Opdyke, 1975), and d-limonene was reported to reduce the sensitivity and delayed hypersensitivity of guinea-pig skin to citral (Hanau *et al.*, 1983; Barbier & Benezra, 1983). Gozsy and Kato (1957) reported that local endothelial phagocytosis is induced in rat skin by d-limonene, and that d-limonene increases phagocytosis in guinea-pig skin *in vivo* and in mouse skin *in vitro* (Gozsy & Kato, 1958) and increases capillary permeability in the guinea-pig (Kato & Gozsy, 1958).

Male BALB/c mice were treated intragastrically with 0, 0.002, 0.008, 0.033, 0.133, 0.531 or 2.125 mg/ml d-limonene daily. After 8 weeks, but not after 4 weeks, the mitogenic responses of spleen cells taken from the treated animals and exposed *in vitro* to concanavalin A and phytohaemagglutinin, which stimulate T-cells, and lipopolysaccharide, which affects B-cells, were found to be suppressed by the d-limonene treatment.

Other animals in groups of 10 were given these same doses of d-limonene daily for 7 days prior to, or 8 days following, immunization with keyhole limpet haemocyanin (KLH). Primary effects on T- and B-cell responses were determined 10 days later.

Secondary effects were determined by re-inoculating the animals with KLH 21 days after the first inoculation and then assaying on day 24. The primary and secondary responses were reported to be suppressed by d-limonene if the animals were exposed to KLH first, but stimulated in treated mice if exposed to KLH after d-limonene exposure.

Histopathological examination of secondary lymphoreticular tissue taken from animals exposed to d-limonene showed significant secondary follicle development and prominent lymphoid nodules and aggregates in the pancreas and intestinal mucosa, which were evident in animals which had received the highest dose of d-limonene (Evans *et al.*, 1987).

Classification of limonene

Limonene (mixed d- and l-isomers) and d-limonene has been classified by ECB in the following way; R10 Xi; R38 R43 N; R50-53

Summary of Limonene data and conclusions for the Spearmint Oil

The most striking effect seen with limonene is the carcinogenic response in the male (not female) rat kidney involving hyaline droplet accumulation, kidney damage and alpha₂μ-globulin. This damage has not been seen in any other mammals such as mice, hamsters, guinea pigs, dogs and monkeys. As the male rat specific alpha₂μ-globulin is considered to play a crucial role in the kidney lesions, they are considered not relevant for human risk assessment and it will be unlikely to occur in any species not producing alpha₂-globulin or a very closely related protein in the large quantities typically in male rat. Moreover, it is not likely that l-carvone the main constituent of Spearmint Oil has the same effect as limonene on male rats as no association to alpha₂μ-globulin were observed in rat kidneys in the 13 week oral toxicity study (study 5, see above). This was supported by the study by Lam, 1988, that indicated that l-carvone did not affect the kidneys.

As limonene is a component of Spearmint Oil, only present in a few percent, the RMS consider that the risk assessment for limonene is cover by the line of arguments used for Spearmint Oil above in section B.6.10.

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

Literature citations

Listing of Test and Study Reports and published Papers not submitted.

Indication of the reason not submitted:

All tests or publications listed below are cited in WHO FOOD ADDITIVE SERIES

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SPEARMINT OIL
Annex B.6: Toxicology and Metabolism

APPENDIX 2 – Estimation of spray operator exposure

ESTIMATION OF SPRAY OPERATOR EXPOSURE - PERCENT ABSORPTION METHOD
PSD (1992) UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM): A USER'S GUIDE

PRODUCT DATA				
1	Name:	Spearmint Oil		
2a	Active ingredient:	L-Carvone		
2b	Concentration:	750 mg/mL or mg/g		
3	Formulation type:	HN		
4a	Main solvent:	Not applicable		
4b	Concentration of solvent:	25 % w/w		
5	Maximum in-use ai concentration:	750 mg/mL		
B	EXPOSURE DURING MIXING AND LOADING			Overestimation for filling fogging apparatus ¹
1a	Container size:	5 litres or kg		
1b	Hand contamination/operation:	0,01 mL or g/operation		
2	Application rate:	0,09 litres or kg product/t		
3	Work rate:	1000 t/day		
4	No. of operations per day:	18		
5	Contamination/day:	0,18 mL or g/day		
6	Protective clothing:	Gloves		
7	Transfer/penetration from gloves:	10 %		
8	Dermal exposure to formulation:	0,018 mL or g/day		
C	EXPOSURE DURING SPRAY APPLICATION			No significant exposure ¹
1	Application technique:	Hot fogging		
2	Application volume:	0,09 litres spray/t		
3	Volume of surface contamination:	0 mL/h		
		hands	trunk	legs
4	Distribution:			
5	Clothing:	none permeable permeable		
6	Penetration/transfer:			
7	Dermal exposure:	0	0	0 mL/h
8	Duration of exposure:	0 h		
9	Total dermal exposure to spray:	0 mL/day		
D	DERMALLY ABSORBED DOSE			Overestimation for filling fogging apparatus ¹
		mixing and loading	spray application	
1	Dermal exposure:	0,018	0 mL/day	
2	Concentration of ai:	750	750 mg/mL	
3	Dermal exposure to ai:	13,5	0 mg/day	
4	Percent absorption:	100	100 %	
5	Absorbed dose:	13,5	0 mg/day	
E	INHALED DOSE (SPRAY APPLICATION)			No significant exposure ¹
1	Inhalation exposure:	0 mL/h		
2	Duration of exposure:	0 h		
3	Concentration of ai:	750 mg/mL		
4	Inhalational exposure to ai:	0 mg/day		
5	Percent absorbed:	100 %		
6	Absorbed dose:	0 mg/day		
F	PREDICTED EXPOSURE			Overestimation for filling fogging apparatus ¹
1	Total dose absorbed:	13,5 mg/day		
2	Operator body weight:	60 kg		
3	Operator exposure:	0,23 mg/kg bw/day		

Note 1: Section 4.4 at http://www.ctb.agro.nl/ctb_files/05829_12.html

Effects of Flavoring and Casing Ingredients on the Toxicity of Mainstream Cigarette Smoke in Rats

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

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

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

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

MATERIALS AND METHODS

Cigarette Design

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

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

TABLE 1
Blend composition of prototype cigarettes

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

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

Cigarette Performance

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

In Vitro Study Design

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

TABLE 2
Ingredients added to test cigarettes in study 1

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

(Continued on next page)

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

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

(Continued on next page)

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

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

(Continued on next page)

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

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

Note. "n" Follows the name of natural source of flavorings and "c" follows the number of chemical substances.

^aChemical Abstract Service registry number.

^bThe Flavor and Extract Manufacturers Association reference number.

^cCode of Federal Regulations reference to Title 21 indicating regulatory status of material.

^dCouncil of Europe reference number.

Inhalation Toxicity Study Design

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

Each group of 30 rats/sex was subdivided into 2 groups: 20 rats/sex scheduled for necropsy immediately after 13 wk

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

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

Smoke Generation and Exposure System

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

TABLE 3
Ingredients added to study 2 test cigarettes

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

Note. "n" Follows the name of natural source of flavorings and "c" follows the number of chemical substances.

^aChemical Abstract Service registry number.

^bThe Flavor and Extract Manufacturer's Association reference number.

^cCode of Federal Regulations reference to Title 21 indicating regulatory status of material.

^dCouncil of Europe reference number.

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

Exposure Atmosphere Characterization

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

Two filters per exposure group per week were chemically analyzed for total nicotine. Nicotine standard reference material (98%) was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). The WTPM:nicotine and CO:nicotine ratios

were calculated for the exposure atmospheres. The concentration of CO in the test and reference atmospheres was determined using Horiba PIR-2000 CO analyzers (Horiba Instruments, Inc., Irvine, CA), monitored by DOS-based computers.

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

Animals and Animal Care

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

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

Body Weight and Clinical Observations

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

Respiratory Function Measurements

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

Carboxyhemoglobin and Plasma Nicotine Determinations

During wk 2 and 10, blood was collected from designated animals at the end of the 1-h smoke exposure. Animals were removed from the exposure unit and bleeding was initiated within ~5 min. The blood samples were obtained from the retro-orbital plexus of carbon dioxide (CO₂)-anesthetized animals into tubes containing potassium ethylenediamine tetraacetic acid (K⁺-EDTA). The sample tubes were immediately placed into an ice bath and maintained under these conditions until analyzed for blood carboxyhemoglobin (COHb). Plasma nicotine was quantitatively determined using gas chromatography/mass spectrometry (GC/MS) with selected ion monitoring.

Clinical Pathology

On the day of the 13-wk interim sacrifice, the rats were anesthetized with ~70% CO₂ in room air and blood samples were obtained from the retro-orbital plexus. One sample was collected in a tube (Monoject, Sherwood Medical, St. Louis, MO) containing K⁺-EDTA for hematologic determinations. Another sample was collected in a tube devoid of anticoagulant but containing a separator gel (Vacutainer, Franklin Lakes, NJ) for serum chemistry analysis. The following parameters were determined using an Abbott Cell-Dyn 3700 (Abbott Diagnostics Systems, Abbott Park, IL) multiparameter hematology instrument: white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (Hb) concentration, volume of packed red cells (VPRC), the red cell indices (mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], and mean corpuscular hemoglobin concentration [MCHC]), platelet count, and WBC differential counts. Results of the differential cell counts were reported as both relative and absolute values. Reticulocytes were stained supravitaly with new methylene blue and enumerated as reticulocytes per

1000 erythrocytes using the Miller disc method (Brecher and Schneiderman, 1950).

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

Necropsy and Tissue Collection

A complete necropsy was done on all 13-wk exposure groups and 13-wk recovery group animals. Rats designated for scheduled sacrifices or sacrificed due to moribund condition were weighed and anesthetized with 70% CO₂ in air, followed by exsanguination before cessation of heartbeat. All abnormalities were recorded on the individual animal necropsy forms. Lungs, liver, kidneys, testes, adrenals, spleen, brain, and heart from all scheduled sacrifice animals were weighed. These organ weights and the body weights at necropsy were used to calculate organ:body weight ratios. In addition, organ:brain weight ratios were calculated. The time from removal of the organ until weighing was minimized to keep tissues moist.

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

Histopathology

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

Evaluation of Cell Proliferation Rates of Respiratory-Tract Tissues

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

Statistical Methods

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

RESULTS

Cigarette Performance

The results of characterization of the test and reference cigarettes for study 1 and study 2 are presented in Tables 4 and 5. These results show that the filler weight and the number of puffs per cigarette, nicotine yield, and nicotine-free dry particulate matter (NFDPM) were comparable for test and reference

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

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

Note. Cig, cigarette.

cigarettes in both studies. The yields of nicotine and NFDPM and the puff count were also comparable. These results are consistent with the negligible differences in the configuration of both prototype cigarettes, which basically consist of the total relative amount of flavor ingredient contained in the test cigarettes (1% or 3% of the filler weight). A comparison of the burning rates in study 1 illustrates that the addition of the ingredients had little, if any effect on the burning characteristics of the test cigarettes.

In Vitro Mutagenicity Assays

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

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

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

Note. Cig, cigarette.

Exposure Atmosphere Characterization

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

Body Weights and Clinical Observations

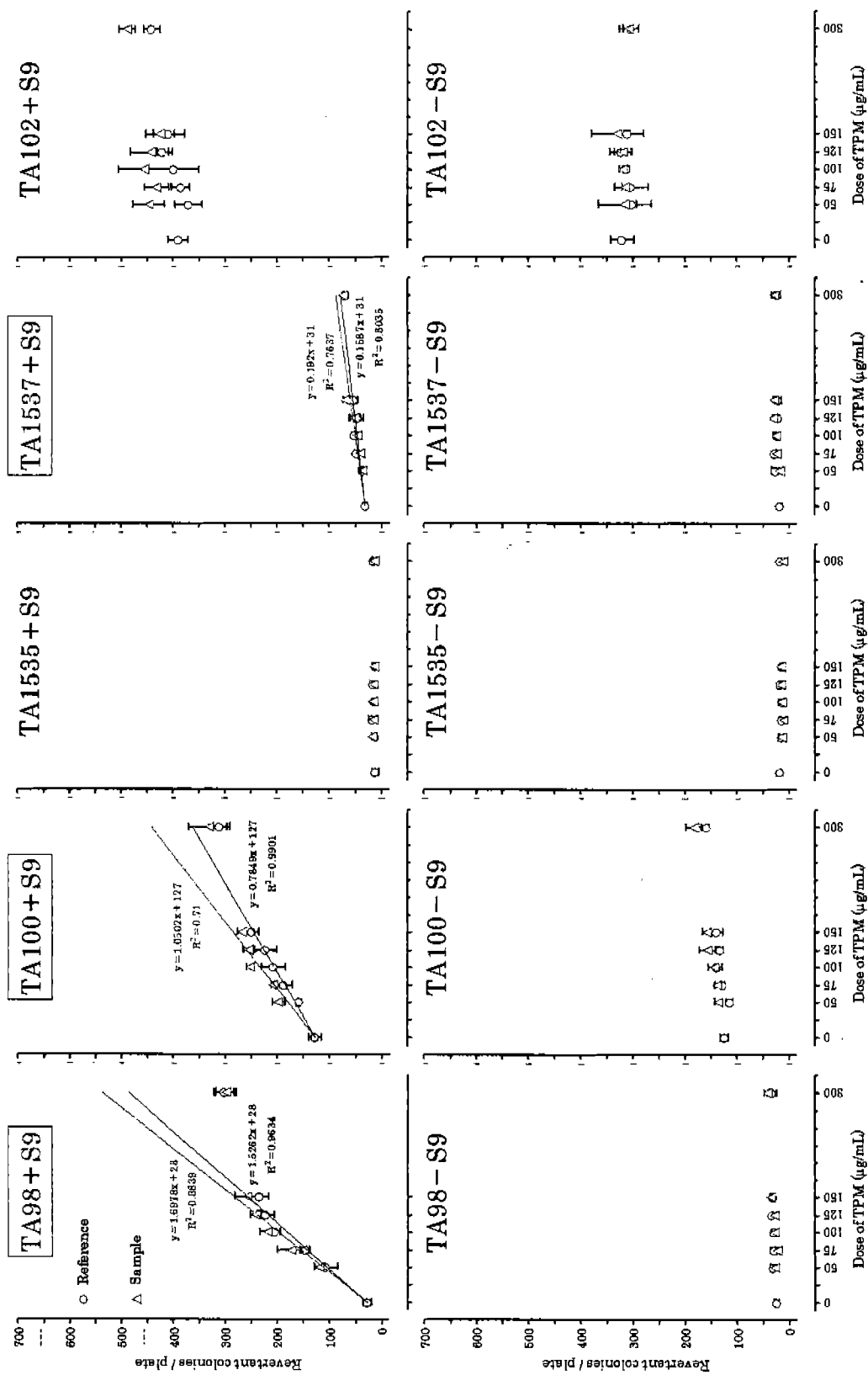
No significant mortality occurred in either study. Exposure-related adverse clinical signs were absent. Clinical observations noted were minor in consequence and low in incidence.

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

smoke-exposed groups were similar to sham control weights during the recovery period of both study 1 and study 2. The only consistent statistical difference in body weight changes between the test and reference cigarette smoke-exposed groups in either study was the decreased mean body weight in males exposed to 0.8 mg/L of reference cigarette smoke during the exposure period of study 1.

Organ Weights

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



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

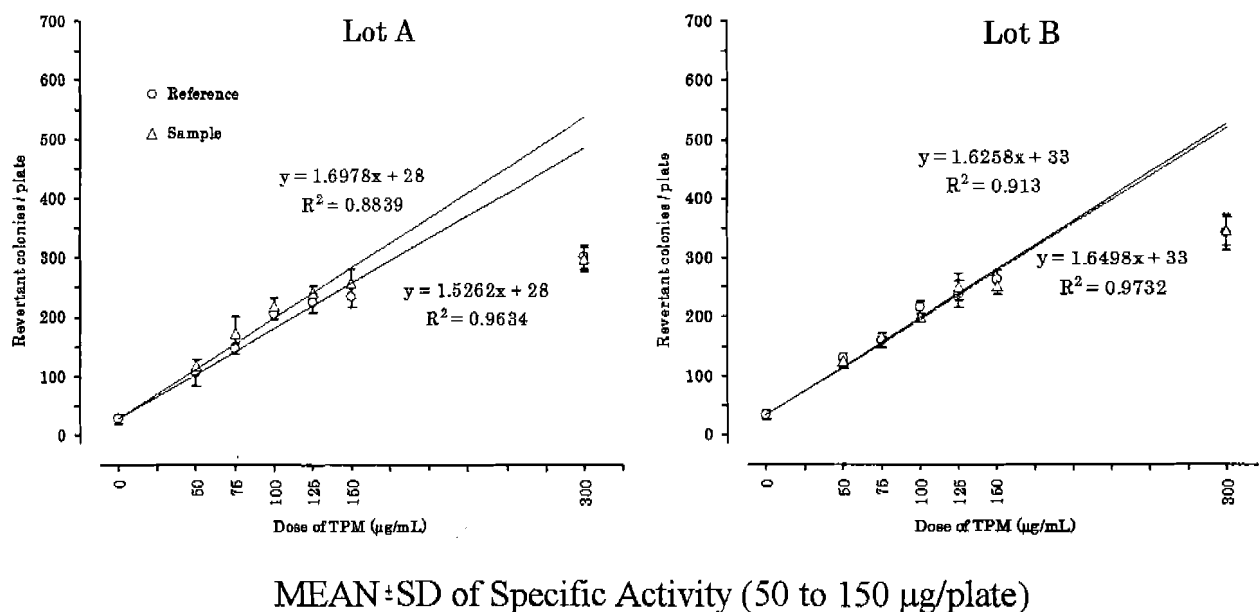


FIG. 2. Ames assay results, study 1 with TA98 metabolic activation.

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

Respiratory Physiology

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

Clinical Pathology

There were occasional statistically significant differences in hematology and clinical chemistry parameters from control values in groups exposed to smoke from test or reference cigarettes in both study 1 and study 2. These differences did not occur in a dose-response pattern and were well within ± 2 standard deviations of historic values for control Sprague-Dawley rats of

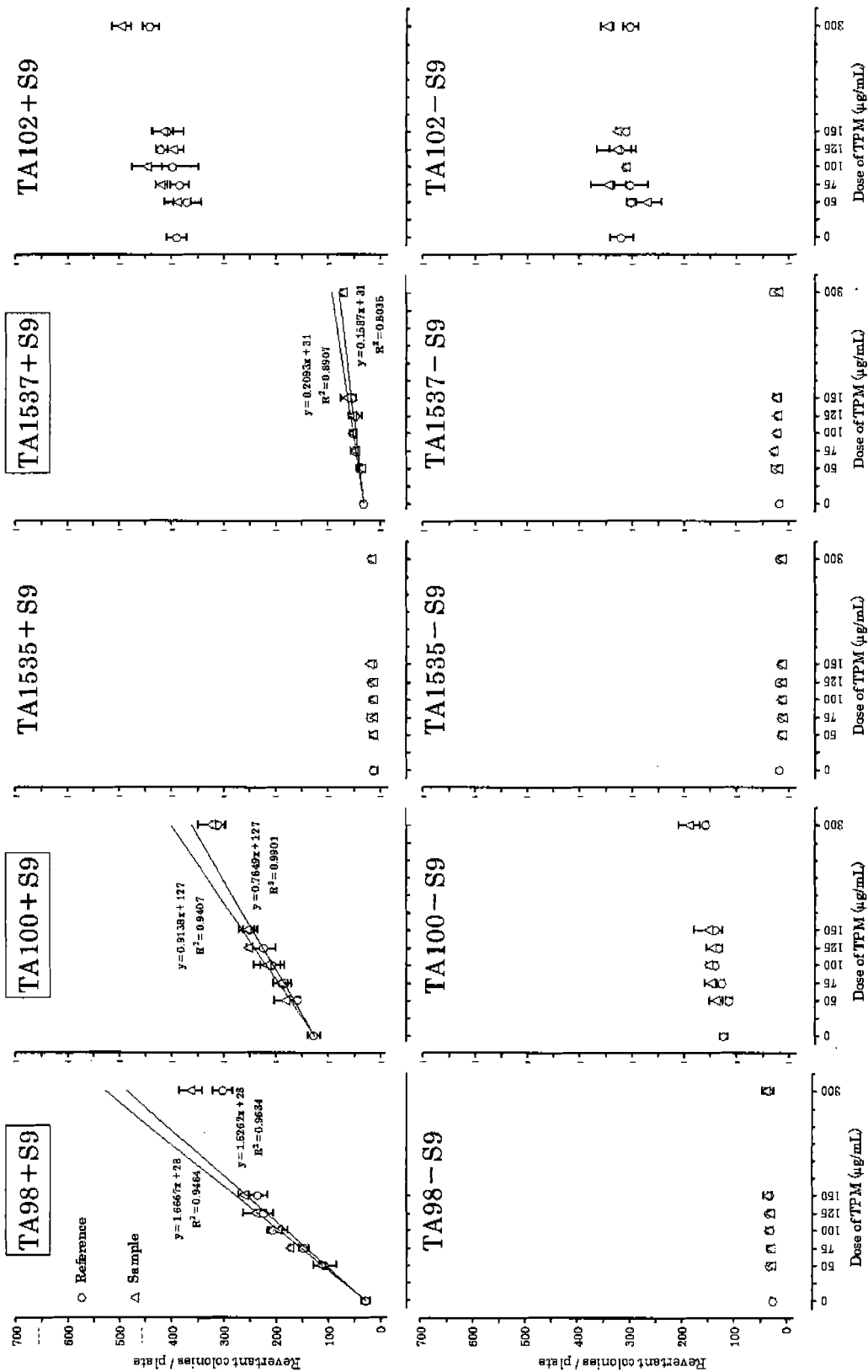
comparable age. There were also statistically significant differences in several hematology and clinical chemistry parameters between groups exposed to similar concentrations of test and reference cigarette smoke. These differences are not considered to be of toxicologic significance, nor were they exposure related.

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

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

Pathology

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



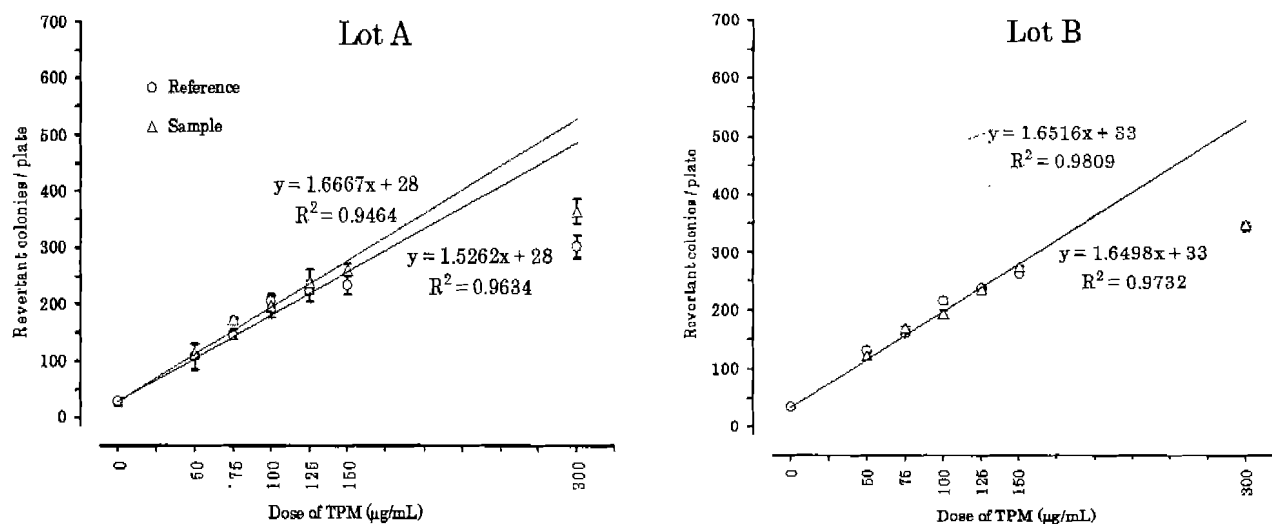
N=2. Only the first lot (Lot A) is indicated in this figure.
 The second lot (Lot B) showed the same tendency as the first lot.

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

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

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

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



MEAN $\pm$ SD of Specific Activity (50 to 150 $\mu\text{g/plate}$)

Reference.....	1576 $\pm$ 141.9	Reference.....	1734 $\pm$ 170.9
Sample.....	1726 $\pm$ 138.6	Sample-1.....	1701 $\pm$ 107.9

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

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

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

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

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

Hyperplasia of respiratory epithelium lining the anterior nasal cavity was present in all rats exposed to 0.8 mg/L in both studies, a few rats exposed to 0.2 mg/L in both studies, and in 3/40 rats exposed to 0.06 mg/L in study 1. Areas most severely and most frequently affected were the distal portions of the nasal and maxillary turbinates in sections of nose just caudal to the incisor teeth. In affected rats, the epithelium in the distal turbinates was up to six cells thick. There was also a clear dose response in the severity of nasal respiratory epithelial hyperplasia, with severity ranging from minimal to moderate. Comparison of incidence and severity data for nasal respiratory epithelial hyperplasia in rats exposed to similar concentrations of smoke from the test and reference cigarettes did not indicate any statistically significant differences in either study. Minimal goblet-cell hyperplasia was observed in the mucosal epithelium lining the median nasal septum in some smoke-exposed and sham control rats. Although not statistically significant compared to concurrent sham controls, the incidence of nasal goblet cell hyperplasia in male rats exposed to the 0.8-mg/L concentration of smoke from the reference cigarette or test cigarette in study 1 were considered to be

toxicologically significant. There was no clear difference in the incidence of goblet cell hyperplasia between groups exposed to similar concentrations of reference and test cigarette smoke in either study.

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

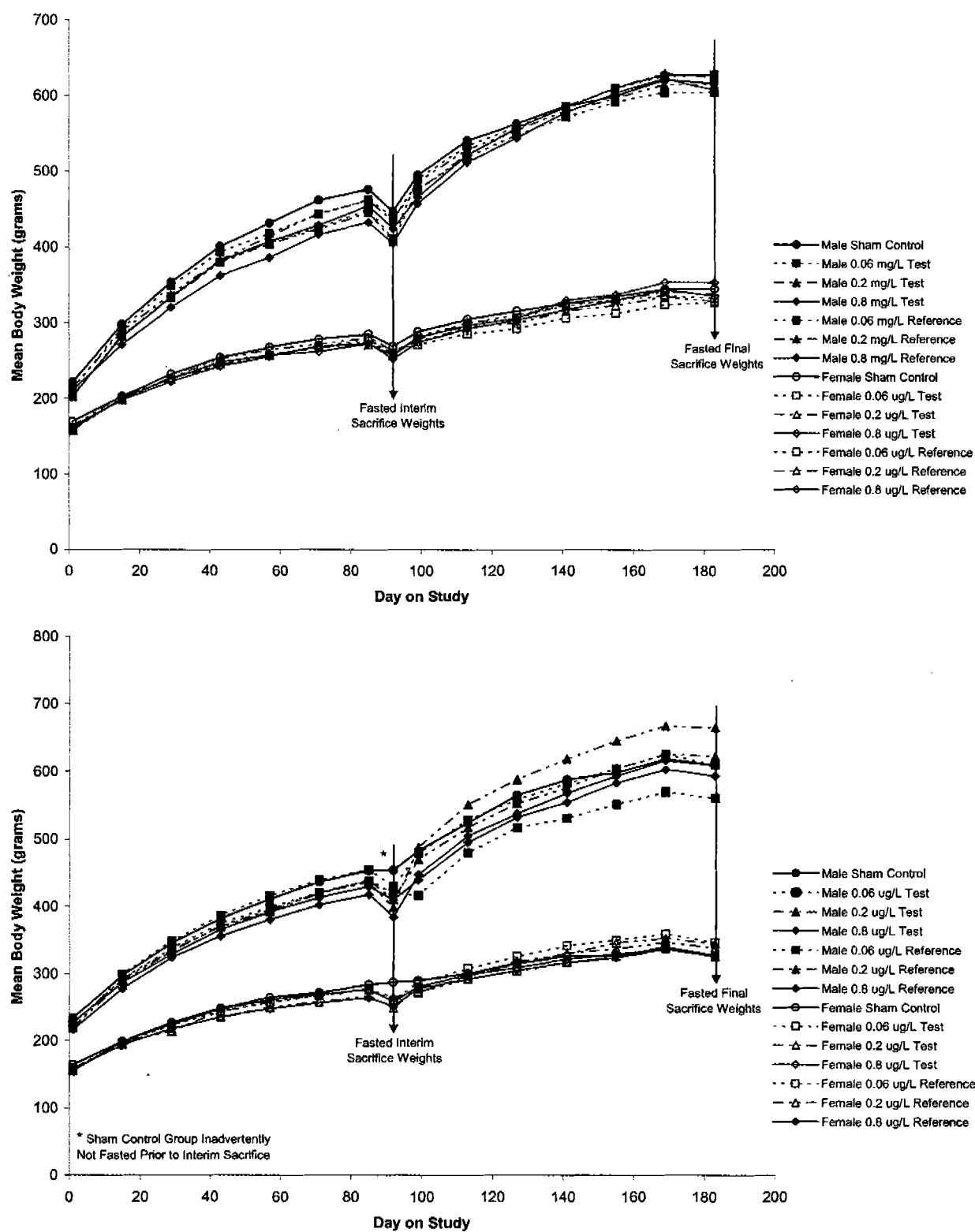


FIG. 5. Body weights, study 1 (top) and study 2 (bottom).

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

		Test			Reference		
	Sham control	0.06 mg WTPM/L	0.2 mg WTPM/L	0.8 mg WTPM/L	0.06 mg WTPM/L	0.2 mg WTPM/L	0.8 mg WTPM/L
Males							
Heart	1.60 ± 0.16	1.48 ± 0.15 ^{a,b}	1.43 ± 0.16 ^{a,c}	1.55 ± 0.15	1.60 ± 0.13	1.57 ± 0.16	1.52 ± 0.15
Kidneys	3.39 ± 0.33	3.17 ± 0.39	2.92 ± 0.30 ^{a,c}	3.05 ± 0.33 ^a	3.38 ± 0.33	3.20 ± 0.31	3.02 ± 0.27 ^a
Lungs	1.95 ± 0.22	1.89 ± 0.17	1.82 ± 0.23 ^c	1.93 ± 0.14	2.02 ± 0.28	1.98 ± 0.26	1.89 ± 0.15
Adrenals	0.066 ± 0.010	0.066 ± 0.012	0.059 ± 0.010	0.064 ± 0.012	0.062 ± 0.007	0.064 ± 0.008	0.063 ± 0.008
Females							
Heart	1.06 ± 0.09	1.02 ± 0.10	1.00 ± 0.10 ^c	1.05 ± 0.12	1.03 ± 0.09	1.07 ± 0.09	1.09 ± 0.12
Kidneys	2.18 ± 0.21	2.02 ± 0.24	1.90 ± 0.19 ^a	1.93 ± 0.18 ^a	2.04 ± 0.21	1.99 ± 0.19 ^a	1.95 ± 0.19 ^a
Lungs	1.53 ± 0.13	1.50 ± 0.13	1.52 ± 0.17 ^c	1.52 ± 0.15	1.55 ± 0.14	1.50 ± 0.17	1.60 ± 0.19
Adrenals	0.080 ± 0.010	0.081 ± 0.011	0.078 ± 0.008	0.082 ± 0.012	0.078 ± 0.008	0.080 ± 0.010	0.081 ± 0.013

^a $p < .05$, Dunnett's t -test of significance, compared to sham control.

^b $p < .05$, Dunnett's t -test of significance, compared to 0.06 reference group.

^c $p < .05$, Dunnett's t -test of significance, compared to 0.2 reference group.

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

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

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

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

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

Note. Severity: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

^aNumber of tissues or animals examined.

^bNumber of diagnoses made.

^c $p < .05$, Kolmogorov-Smirnov test, compared to 0.06-mg/L reference group.

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

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

Note. Severity: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

^aNumber of tissues or animals examined.

^bNumber of diagnoses made.

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

Evaluation of Cell Proliferation Rates

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

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

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

DISCUSSION

The studies described here were designed to evaluate the potential influence of ingredients on the chemical composition and the biological activity of mainstream cigarette smoke. Test cigarettes containing flavorings or casings were analyzed and compared against reference cigarettes identical except produced without flavors or casings. The configuration and ISO-condition

tar, nicotine, and CO yields of all cigarettes investigated are representative of American blend cigarettes. Both test and reference cigarettes had the same tobacco blend and humectant composition (glycerine plus water) and were prepared by the same manufacturing process. Similarly, identical nontobacco materials (NTM) were used throughout. The weight of the filler remained constant between test and reference cigarettes. These studies illustrate that the application of 165 low-use flavoring or 8 high-use flavoring or casing ingredients had little, if any, observable effect on the deliveries or physical parameters of the cigarettes.

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

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

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

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

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

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

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

The effects of inhaled cigarette smoke on the respiratory tract of rats in both the studies described herein are similar to those described in a number of previously reported cigarette smoke inhalation studies in rats (Dalbey et al., 1980; Gaworski et al.,

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

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Preface

The List of Undesirable Substances (LOUS) was established by the Danish Environmental Protection Agency (EPA) as a guide for enterprises. It addresses chemical substances of concern, based on their hazardous properties and the volumes used in Denmark. The latest version of LOUS from 2009 includes 40 chemical substances or groups of substances (DK EPA 2010).

During the period 2012-2015, all substances listed on LOUS have been surveyed and further need for risk management measures will be evaluated. In certain cases, implementation projects have been launched to achieve the goals laid down in the strategies for each of these substances/substance groups.

The present project "Evaluation of selected sensitising fragrance substances" was initiated as a LOUS follow-up project by the Danish EPA. The objective of this study was to evaluate selected fragrance substances in relation to the classification criteria for strong sensitisers (Category 1A sensitisers) according to the CLP Regulation on classification, labelling and packaging of substances and mixtures (EC no. 1272/2008)¹.

The project was carried out from July to November 2015 at the National Food Institute, Technical University of Denmark.

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¹ REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006, with amendments M1-M9 and corrigenda C1-C2, 1 June 2015. <http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02008R1272-20150601&from=en>

Summary and conclusion

Fragrances are widely used in many different types of consumer products. Therefore, the general population can be exposed to fragrances from many different sources and consequently can have a substantial exposure despite the fact that fragrances most often are used in a relatively low concentration in individual consumer products.

Many fragrances have been shown to cause harmful effects to human health. Skin sensitisation (contact allergy) is identified as the critical effect for a wide range of fragrances. Many fragrances are already classified as skin sensitisers (Skin Sens Cat 1) according to the CLP Regulation (EC no. 1272/2008). The CLP criteria for classification of skin sensitisers were revised in 2011 and now provide possibility for sub-categorising skin sensitisers in two sub-categories, sub-category 1A (strong sensitisers) or 1B (other skin sensitisers). Some of the fragrances that are already classified as skin sensitisers in Category 1 may possibly fulfil the CLP criteria for classification as strong sensitisers in sub-category 1A.

A classification of a specific substance in sub-category 1A implies that classification and labelling of mixtures containing the substance is required at a lower concentration (factor 10) compared to skin sensitisers in Category 1. The more stringent labelling requirements for sub-category 1A sensitisers will apply for mixtures under the scope of the CLP regulation such as e.g., washing and cleaning products. Consequently, the labelling may increase the protection of users (workers, consumers) as it will allow sensitised individuals to take precautionary measures to prevent direct skin contact with a product containing a strong sensitiser.

The purpose of this project was to retrieve and review the available data for 42 selected fragrances already classified as skin sensitisers in Category 1 (harmonised and/or notified classification) in order to assess whether these substances fulfil the criteria for classification in sub-category 1A. The 42 fragrances (substances / natural extracts), hereafter referred to as substances, were selected by the Danish EPA based on information in a LOUS survey of selected fragrances (DK EPA, 2015) and in an SCCS opinion on fragrance allergens in cosmetic products (SCCS, 2012), as well as on other criteria set up by the Danish EPA, as described below in Chapter 2. The list of substances for evaluation as provided by the Danish EPA is presented in Appendix 2.

The project was divided in two phases:

In Phase 1, relevant information was retrieved for all the selected 42 substances as described in Chapter 3, section 3.1. The relevant information for the purpose of the screening in this project is human data (primarily patch tests in unselected and/or selected dermatitis patients) and animal data (local lymph node assay, guinea pig maximization test and the Buehler assay). Then, a preliminary evaluation of the relevant data was performed in order to identify possible sub-category 1A candidates. Therefore, a thorough evaluation of the retrieved data was not performed in Phase 1.

In Phase 2, a more detailed assessment of the data for selected sub-category 1A candidates was performed as described in Chapter 3, section 3.2. The quality of the data was assessed according to the Klimisch criteria (Klimisch et al., 1997) as described in section 3.2.1. The 'reliable' data (assigned score 1 or 2) were then assessed against the CLP classification criteria for skin sensitisation with special focus on whether classification in sub-category 1A is justified. Other data

(assigned score 4) were also included in the assessment. Not 'reliable' data (assigned score 3) were not included in the assessment. The CLP classification criteria for classification in sub-category 1A are summarised in section 3.2.2.

Based on the preliminary assessment in [Phase 1](#), each of the 42 selected substances was given a priority 1 or 2 as described in Chapter 4. Priority 1 was given to those substances which were considered as possible sub-category 1A candidates, in total 20 substances (listed in the first table in Chapter 4). Priority 2 was given to those substances which were not considered as possible sub-category 1A candidates, in total 22 substances. The justification for the prioritisation of the substances as 1 or 2 is presented in Appendix 3.

Among the 20 identified possible sub-category 1A candidates, a further prioritisation was performed for the selection of the Phase 2 substances. The highest priority was given to the substances identified in the SCCS opinion (SCCS, 2012) as established contact allergens in humans and considered to be of special concern (marked with an 'X' in the right column of the table in Chapter 4) and/or based on the clinical experience from the National Allergy Research Centre. The 11 substances taken forward to Phase 2 are listed in the table below:

Substances	CAS RN	Substances of special concern according to SCCS (2012)
Citral	5392-40-5	X
Cinnamaldehyde	104-55-2	X
Cinnamyl alcohol	104-54-1	X
Coumarin	91-64-5	X
Eugenol	97-53-0	X
Farnesol	4602-84-0	X
Geraniol	106-24-1	X
7-Hydroxycitronellal	107-75-5	X
Methyl oct-2-ynoate	111-12-6	
<i>Cinnamomum cassia</i> leaf oil / <i>Cinnamomum zeylanicum</i>, ext.	8007-80-5 / 84649-98-9	
<i>Evernia prunastri</i>, ext.	90028-68-5	X

The results of the detailed assessment performed for the 11 substances selected for [Phase 2](#) are presented in Chapter 5 as a summary of the available data, a comparison with CLP Regulation criteria for classification in sub-category 1A, and a conclusion on classification for each substance. Appendices 4-14 present the full overview of the available studies/data for the substances and the evaluation of their skin sensitising potential. For all 11 substances, a classification as a skin sensitiser in sub-category 1A is justified based on the available data.

For nine of the substances, sub-category 1A is justified based on human patch test data i.e. frequencies in unselected and/or selected dermatitis patients and/or a high number of cases. For methyl oct-2-ynoate, sub-category 1A is justified primarily based on non-human data (very low EC3 values in the two LLNAs and results from GPMT and Buehler tests, and supported by human evidence from HRIPT studies). For *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext., sub-category 1A is justified based on their constituents by read across to the major compounds such

as cinnamaldehyde (for Cassia bark extract, Cassia oil, Cinnamon bark extract and Cinnamon bark oil) and eugenol (for Cinnamon leaf oil).

The experiences from the assessments in this project are discussed in Chapter 6. In general, a decision whether a classification of a substance as a skin sensitiser in sub-category 1A is justified or not is based on the available human data. This classification is generally not supported by the available non-human data available for the 11 substances assessed in this project. However, as a substance can be classified in sub-category 1A on the basis of reliable and good quality evidence from human cases or epidemiological studies and/or observations from appropriate studies in experimental animals it seems clear that sub-category 1A is justified based on the available human data.

As illustrated by one of the 11 Phase 2 substances, methyl oct-2-ynate, sub-category 1A can also be justified primarily based on non-human data, in this case based on a very low EC3 value in the two LLNAs, and the results from GPMT and Buehler tests (and in this case, supported by human evidence from HRIPT studies).

As illustrated by another of the 11 Phase 2 substances, *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext., read across to major constituents in the fragrance could justify a classification in sub-category 1A.

For seven of the 42 selected substances, the available data were too limited for an indication as a possible sub-category 1A candidate. Thus, it cannot be excluded whether these substances would turn out to be sub-category 1A candidates should additional human studies and/or non-human data become available.

A relatively large proportion of the available studies have only been available from secondary sources, mainly attributed to unpublished data by the industry. Inclusion of these studies in this project is justified due to the purpose of the project, i.e. a screening of the available data for a preliminary assessment whether the criteria for classification in sub-category 1A according to the CLP criteria are fulfilled.

Secondary sources have a reliability score 4 “not assignable” according to the reliability criteria proposed by Klimisch et al. (1997). For a genuine assessment of studies only available from secondary sources, the original study report should be available for the assessment.

Conclusion:

For all 11 substances selected for detailed assessment in Phase 2, a classification as a skin sensitiser in sub-category 1A is justified based on the available data. Based on the assessments in this project, a decision whether a classification of a substance as a skin sensitiser in sub-category 1A is justified is generally based on the available human patch test data.

One of the elements in the criteria for classification in sub-category 1A based on human patch test data is ‘relatively low exposure’. For the 11 substances, relatively low exposure from use as fragrance in cosmetics and in other consumer products has been evaluated based on the IFRA standard limit of each substance in each of the 11 product categories for dermal sensitisation which have been established by IFRA based on quantitative risk assessment (QRA). The IFRA standard limits are still under evaluation. Revisions of the QRA approach might result in changes of the current IFRA standard limits and could thus have an impact on the classification as a skin sensitiser in sub-category 1A for the 11 substances.

A relatively large proportion of the cited studies have only been available from secondary sources, mainly attributed to unpublished data by the industry. For a genuine assessment of such studies, the original study reports should be available for the assessment. Thus, it cannot be excluded whether the outcome of the assessments performed in this project could turn out differently should the original study reports become available for the final assessment.

Sammenfatning og konklusion

Duftstoffer er meget udbredt i mange forskellige typer af forbrugerprodukter. Den generelle befolkning kan således blive udsat for duftstoffer fra mange forskellige kilder og kan derfor have en væsentlig udsættelse herfor til trods for, at duftstoffer oftest anvendes i en relativt lav koncentration i de enkelte forbrugerprodukter.

Mange duftstoffer har vist sig at kunne være skadelige for menneskers sundhed. Hudsensibilisering (kontaktallergi) er blevet identificeret som den kritiske effekt for en lang række duftstoffer. Mange duftstoffer er allerede klassificeret som hudsensibiliserende (Skin Sens Kat 1) i henhold til CLP-forordningen (EF nr. 1272/2008). CLP-kriterierne for klassificering af hudsensibiliserende stoffer blev revideret i 2011 og giver nu mulighed for at klassificere hudsensibiliserende stoffer i to subkategorier, subkategori 1A (stærkt sensibiliserende stoffer) eller 1B (andre hudsensibiliserende stoffer). Nogle af de duftstoffer, der allerede er klassificeret som hudsensibiliserende i kategori 1, kan muligvis opfylde CLP-kriterierne for klassificering som stærkt sensibiliserende i subkategori 1A.

Klassificering af et specifikt stof i subkategori 1A vil medføre, at kemiske blandinger (produkter) indeholdende stoffet skal klassificeres og mærkes ved en lavere koncentration (faktor 10 lavere) i forhold til stoffer klassificeret i kategori 1. De skærpede mærkningskrav for stærkt sensibiliserende stoffer (subkategori 1A) gælder for produkter, der er omfattet af CLP forordningens mærkningskrav som f.eks. vaske- og rengøringsmidler. Mærkningen vil således kunne øge beskyttelsen af brugerne (arbejdere, forbrugere), da mærkningen vil kunne gøre det muligt for sensibiliserede individer at tage forholdsregler for at undgå direkte hudkontakt med et produkt, der indeholder et stærkt sensibiliserende stof.

Formålet med dette projekt var at søge og vurdere de tilgængelige data for 42 udvalgte duftstoffer, som allerede er klassificeret som hudsensibiliserende i kategori 1 (harmoniseret og / eller notificeret klassificering) med henblik på at vurdere, om disse stoffer kan opfylde kriterierne for klassificering i subkategori 1A baseret på de tilgængelige data.

De 42 duftstoffer (stoffer / naturlige ekstrakter), efterfølgende benævnt stoffer, blev udvalgt af Miljøstyrelsen baseret på oplysninger i et LOUS kortlægningsprojekt af udvalgte duftstoffer (DK EPA, 2015) og i en SCCS opinion om allergene duftstoffer i kosmetiske produkter (SCCS, 2012), såvel som på andre kriterier sat af Miljøstyrelsen, som beskrevet nedenfor i kapitel 2. Listen over de udvalgte stoffer er præsenteret i Appendix 2.

Projektet var opdelt i to faser:

I fase 1 blev der indsamlet relevante oplysninger for alle de udvalgte 42 stoffer, som beskrevet i kapitel 3, afsnit 3.1. Relevante oplysninger med henblik på screeningen i dette projekt er humane data (primært lappetest i ikke-selekterede og / eller selekterede dermatitis patienter) og data fra studier i dyreforsøg (local lymph node assay, guinea pig maximization test og Buehler assay). Derefter blev der lavet en foreløbig vurdering af de relevante data med henblik på at identificere mulige subkategori 1A kandidater. Der er således i fase 1 ikke foretaget en grundig evaluering af de indsamlede data.

I fase 2 blev der foretaget en mere detaljeret vurdering af data for de udvalgte subkategori 1A kandidater, som beskrevet i kapitel 3, afsnit 3.2. Kvaliteten af data blev vurderet i henhold til

Klimisch kriterierne (Klimisch et al., 1997), som beskrevet i afsnit 3.2.1. 'Reliable' data (tildelt score 1 eller 2) blev derefter vurderet i forhold til CLP kriterierne for hudsensibilisering med særligt fokus på, hvorvidt klassificering i subkategori 1A er berettiget. 'Other data' (tildelt score 4) blev også inkluderet i vurderingen. Not 'reliable' data (tildelt score 3), blev ikke medtaget i vurderingen. CLP kriterierne for klassificering i subkategori 1A er sammenfattet i afsnit 3.2.2.

På baggrund af den foreløbige vurdering i fase 1 fik hvert enkelt af de 42 udvalgte stoffer en prioritet 1 eller 2, som beskrevet i kapitel 4. De stoffer, der blev vurderet som mulige subkategori 1A kandidater, fik en prioritet 1, i alt 20 stoffer (præsenteret i den første tabel i kapitel 4). De stoffer, der ikke blev vurderet som mulige subkategori 1A kandidater, fik en prioritet 2, i alt 22 stoffer. Begrundelsen for prioriteringen af stofferne er præsenteret i Appendix 3.

Blandt de 20 stoffer, der blev vurderet som mulige subkategori 1A kandidater, blev der foretaget en yderligere prioritering med hensyn til udvælgelsen af fase 2 stoffer. Den højeste prioritet blev givet til de stoffer, som i SCCS opinion (SCCS, 2012) er blevet udpeget som erkendte kontaktallergener hos mennesker og dermed betragtes som værende særligt bekymrende (markeret med et 'X' i højre kolonne i tabellen i kapitel 4), og/eller baseret på klinisk erfaringer fra Videncenter for Allergi. De 11 stoffer udvalgt til fase 2 er præsenteret i den efterfølgende tabel:

Stof	CAS RN	Stoffer som er særligt bekymrende (SCCS, 2012)
Citral	5392-40-5	X
Cinnamaldehyd	104-55-2	X
Cinnamyl alkohol	104-54-1	X
Coumarin	91-64-5	X
Eugenol	97-53-0	X
Farnesol	4602-84-0	X
Geraniol	106-24-1	X
7-Hydroxycitronellal	107-75-5	X
Methyl oct-2-ynoat	111-12-6	
<i>Cinnamomum cassia</i> leaf oil / <i>Cinnamomum zeylanicum</i>, ext.	8007-80-5 / 84649-98-9	
<i>Evernia prunastri</i>, ext.	90028-68-5	X

Resultaterne af den detaljerede vurdering udført for de 11 stoffer udvalgt til fase 2 er præsenteret i kapitel 5 i form af et sammendrag af de tilgængelige data, en sammenligning med CLP kriterierne for klassificering i subkategori 1A, og en konklusion for klassificering af hvert enkelt stof. I Appendix 4-14 er samlet de fulde vurderinger for de enkelte stoffer. For alle 11 stoffer er en klassificering som hudsensibiliserende i subkategori 1A begrundet på grundlag af de tilgængelige data.

For ni af stofferne er subkategori 1A begrundet baseret på data fra humane lappetest data dvs. frekvenser i ikke-selektede og/eller selektede dermatitis patienter og/eller et højt antal tilfælde. For methyl oct-2-ynat er subkategori 1A primært begrundet baseret på data fra dyreforsøg (meget lave EC3 værdier i de to LLNA tests samt resultater fra GPMT og Buehler tests, og støttet af resultater HRIPT studier). For *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext., er

subkategori 1A begrundet baseret på read across til hovedindholdsstofferne såsom cinnamaldehyd (for Cassia bark ekstrakt, Cassia olie, kanel bark ekstrakt og kanel bark olie) og eugenol (for Cinnamon blade).

Erfaringerne fra vurderingerne i dette projekt er diskuteret i kapitel 6. Generelt er vurderingen om, hvorvidt en klassificering af et stof som hudsensibiliserende i subkategori 1A er begrundet eller ej, baseret på de tilgængelige humane data. Denne klassificering er generelt ikke understøttet af de tilgængelige data fra dyreforsøg for de 11 stoffer, der er vurderet i dette projekt. Da et stof kan klassificeres i subkategori 1A på grundlag af pålidelige humane data af god kvalitet og/eller data fra dyreforsøg, så er subkategori 1A berettiget på grundlag af de tilgængelige humane data.

Som illustreret med et af de 11 fase 2 stoffer, methyl oct-2-ynat, så kan klassificering i subkategori 1A også begrundes primært baseret på data fra dyreforsøg, i dette tilfælde baseret på en meget lav EC3 værdi i de to LLNA tests samt resultaterne fra GPMT og Buehler tests (og i dette tilfælde, støttet af dokumentation fra fra HRIPT studier).

Som illustreret med et andet af de 11 fase 2 stoffer, *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext., så kan klassificering i subkategori 1A begrundes baseret på read across til hovedindholdsstofferne i duftstoffet.

For syv af de 42 udvalgte stoffer blev de tilgængelige data vurderet til at være for begrænsede til en vurdering af stoffet som en mulig subkategori 1A kandidat. Det kan således ikke udelukkes, hvorvidt disse stoffer ville kunne vise sig at være subkategori 1A kandidater, hvis yderligere humane data og/eller data fra dyreforsøg bliver tilgængelige.

En forholdsvis stor del af de tilgængelige studier har kun været tilgængelige fra sekundære kilder, primært i form af upublicerede data fra industrien. Inddragelse af disse studier i dette projekt er berettiget som følge af formålet med projektet, dvs. en screening af de tilgængelige data med henblik på en indledende vurdering af, hvorvidt CLP kriterierne for klassificering i subkategori 1A er opfyldt.

Sekundære kilder har en 'reliability' score 4 "not assignable" i henhold til Klimisch kriterierne (Klimisch et al., 1997). For at kunne foretage en uvildig vurdering af studierne citeret fra sekundære kilder, så bør man have adgang til den originale studierapport.

Konklusion:

For alle 11 stoffer udvalgt til den detaljerede vurdering i fase 2 er en klassificering som hudsensibiliserende i subkategori 1A berettiget på grundlag af de tilgængelige data.

Baseret på vurderingerne i dette projekt så er vurderingen om, hvorvidt en klassificering af et stof som hudsensibiliserende i subkategori 1A er begrundet eller ej, generelt baseret på de tilgængelige humane patch test data.

Et af elementerne i kriterierne for klassificering i subkategori 1A baseret på humane patch test data er 'relativ lav eksponering'. For de 11 stoffer er relativ lav eksponering ved anvendelse som duftstof i kosmetik og andre forbrugerprodukter evalueret baseret på IFRA standard grænser for hvert enkelt stof i hver enkelt af de 11 produktkategorier for dermal sensibilisering som IFRA har opstillet på baggrund af kvantitativ risikovurdering (QRA). IFRA standard grænserne baseret på kvantitativ risikovurdering (QRA) er stadig under evaluering. Revidering af QRA tilgangen kunne således medføre ændringer i de nuværende IFRA standard grænser og kunne dermed influere på klassificeringen som hudsensibiliserende i subkategori 1A for de 11 stoffer.

En forholdsvis stor del af de citerede studier har kun været tilgængelige fra sekundære kilder, primært i form af upublicerede data fra industrien. For at kunne foretage en uvildig vurdering af studierne citeret fra sekundære kilder, så bør man have adgang til den originale studierapport. Det kan således ikke udelukkes, at vurderingerne i dette projekt kunne falde anderledes ud, hvis de originale studierapporter bliver tilgængelige for den endelige vurdering.

1. Introduction

Fragrances are widely used in many different types of consumer products. Therefore, the general population can be exposed to fragrances from many different sources and the exposure may be substantial despite the fact that fragrances most often are used in relatively low concentrations in individual consumer products.

Many fragrances have been shown to cause harmful effects to human health as well as to the environment. In relation to human health, skin sensitisation (contact allergy) is identified as the critical effect for a wide range of fragrances. Many fragrances are already classified as skin sensitisers (Skin Sens Cat 1) according to the CLP Regulation on classification, labelling and packaging of substances and mixtures (EC no. 1272/2008).

The CLP criteria for classification of skin sensitisers were revised in 2011 and now provide possibility for sub-categorising sensitisers in Category 1 in two sub-categories: Sub-category 1A or 1B. Sub-category 1A comprises sensitisers for which exposure to a low amount of the substance may cause a high frequency of occurrence of skin sensitisation in humans and/or a high potency in animals (strong sensitisers), while sub-category 1B comprises other skin sensitisers. Sub-categorisation of skin sensitisers in either category 1A or 1B should thus be done if justified by the available data. If the data do not allow for sub-categorisation, the substance should be classified in Category 1 according to the criteria specified.

Some of the fragrances that are already classified as skin sensitisers in Category 1 and other fragrances which are known to cause skin sensitisation may possibly fulfil the CLP criteria for classification in sub-category 1A. A classification of a specific substance in sub-category 1A implies that classification and labelling of mixtures containing the substance is required at a lower concentration compared to skin sensitisers in Category 1. The hazard statement H317 (May cause an allergic skin reaction) will thus be required at concentrations $\geq 0.1\%$ and labelling with the supplemental hazard statement EUH208 ("Contains <name of sensitising substance>. May produce an allergic reaction") will be required at concentrations $\geq 0.01\%$ for strong sensitisers (sub-category 1A) according to the CLP Regulation. These concentration limits are a factor 10 lower than the concentration limits for classification and labelling of mixtures containing sensitisers in Category 1.

The more stringent labelling requirements for strong sensitisers (sub-category 1A) will apply for mixtures under the scope of the CLP regulation such as e.g., washing and cleaning products. Consequently, the labelling may increase the protection of users (workers, consumers) as it will allow sensitised individuals to take precautionary measures to prevent direct skin contact with a product containing a strong sensitiser.

In an SCCS (EU Scientific Committee on Consumer Safety) opinion on fragrance allergens in cosmetic products it is mentioned that based on data from the fragrance industry, 80 % of the total fragrance chemical volume is used in cosmetics and 20 % in household products (SCCS, 2012). It should be noted that cosmetic products are not subject to classification and labelling under the scope of the CLP regulation (EC no. 1223/2009). However, according to the Cosmetic Regulation *"Perfume and aromatic compositions and their raw materials shall be referred to by the terms 'parfum' or 'aroma'. Moreover, the presence of substances, the mention of which is required under the column 'Other' in Annex III, shall be indicated in the list of ingredients in addition to the terms*

parfum or aroma.” Annex III (entries 67-92) includes 26 fragrances which have been identified as human allergens and must be indicated in the list of ingredients when the concentration of the fragrance exceeds 0.001 % in leave-on products and 0.01 % in rinse-off products.

As part of the Danish Environmental Protection Agency’s (Danish EPA) survey of the ‘List of Undesirable Substances’ (LOUS) a survey of selected fragrances has been carried out (DK EPA, 2015). In that survey, 15 substances with a classification (harmonised/notified) for their skin sensitising potential in Category 1 according to the CLP criteria were identified.

Furthermore, 82 substances / natural extracts were identified in the SCCS opinion on fragrance allergens in cosmetic products (SCCS, 2012) as established contact allergens in humans.

Based on the LOUS review and the SCCS (2012) opinion the Danish EPA has selected 42 substances / natural extracts (hereafter referred to as substances) for a screening of the available data regarding skin sensitisation, see Chapter 2.

The purpose of this project was to retrieve and review the available data for 42 selected substances in order to assess whether the selected substances fulfil the criteria for classification in sub-category 1A according to the CLP criteria.

The project was divided in two phases:

- Phase 1: Screening of the available data for the 42 selected substances, for a preliminary evaluation whether the data justify a classification as a skin sensitiser in sub-category 1A (strong sensitisers).
- Phase 2: A more detailed assessment of the data for those substances identified in Phase 1 as possible sub-category 1A candidates.

2. Selection of substances

The Danish EPA has selected 42 substances for a screening of the available data regarding skin sensitisation. The substances were selected based on information in the LOUS report (DK EPA, 2015) and in the SCCS opinion (SCCS, 2012) as described below, as well as on other criteria set up by the Danish EPA.

2.1 Selection of substances from the LOUS survey report

As part of the Danish EPA's survey of the 'List of Undesirable Substances' (LOUS) a survey of selected fragrances has been carried out (DK EPA, 2015). In that survey "*... it was decided to focus on harmonised classified substances registered under REACH AND included in the list of fragrance substances developed by IFRA*", the International Fragrance Association.

In total, 44 substances with a harmonised classification (health and/or environment) were included in the IFRA list and these 44 substances (appear on a blue background in Appendix 1 of the LOUS report) were included for further assessment in the LOUS survey report. The group of the 44 substances both includes substances which are associated with a scent and substances which are used in fragrance mixtures to keep the fragrance liquid (solvent), preserve the fragrance (and therefore also the scent), adjuvants (i.e. substances that modifies the effect of other substances), and pigments which are applied in order to achieve a certain wanted colour etc.

Among the 44 fragrance substances in the LOUS survey report 15 of the substances were classified as skin sensitisers in Category 1 (either by a harmonised classification or a self-classification).

2.2 Selection of substances from the SCCS (2012) opinion

The EU Scientific Committee on Consumer Safety (SCCS) has published an opinion on fragrance allergens in cosmetic products (SCCS, 2012). In this opinion, 82 substances were identified as established contact allergens in humans, listed in Table 7-1 (54 individual fragrance chemicals) and 7-5 (28 natural extracts) in the opinion.

Seven of the 82 substances are among the 15 substances identified in the LOUS report with a notified classification for their skin sensitising potential.

2.3 The substances selected for this project

Among the 15 substances identified in the LOUS report with a classification (harmonised and/or notified) for their skin sensitising potential and the 82 substances identified in the SCCS (2012) opinion as established contact allergens in humans (a total of 90 substances), the Danish EPA has selected 42 substances (56 CAS numbers) for the screening in this project. All of the 42 substances either have a harmonised classification for skin sensitisation or have been self-classified for skin sensitisation by companies placing the substance on the market in the EU.

The 42 substances were selected based on the following criteria:

- High tonnage (> 100 tonnes/year) and large extent of agreement of sensitising properties:

The substance is registered under REACH and is classified as a skin sensitiser (either a harmonised classification or a self-classification). In case of self-classification >75% of the notifying companies have self-classified as a skin sensitiser (Skin Sens Cat 1) *or*

- Low tonnage but full agreement on sensitising properties:
The substance is not registered under REACH but has a harmonised classification as a skin sensitiser (Skin Sens Cat 1) *or*
- Low tonnage but indication of the substance being a strong sensitiser:
The substance is not registered under REACH but one or more companies have self-classified the substance as a strong sensitiser (Skin Sens Cat 1A) *or*
- None of the above criteria are fulfilled but the substance is identified as a 'high risk substance' in relation to sensitisation by SCCS (2012): The substance has been identified to be of special concern by SCCS (2012) due to a high number of reported positive human cases of sensitisation

The remaining 48 substances that were not selected for further evaluation in Phase 1 included substances that were:

- Low tonnage (substances not registered under REACH) and which did not fulfil any of the above criteria
- High tonnage but no or very low indication of sensitising properties (substances registered under REACH and for which none or only a few companies have notified a self-classification for skin sensitisation).

The 42 selected substances are listed in the following table:

Substances	CAS RN	Substances of special concern according to SCCS (2012) ²
Butyl methacrylate	97-88-1	
Benzaldehyde	100-52-7	
Citral	5392-40-5	X
(R)-p-Mentha-1,8-diene (d-limonene)	5989-27-5	
(S)-p-Mentha-1,8-diene (l-limonene)	5989-54-8	
2-Methyl-4-phenylpentanol	92585-24-5	
A mixture of: trans-4-acetoxy-4-methyl-2-propyl-tetrahydro-2H-pyran; cis-4-acetoxy-4-methyl-2-propyl-tetrahydro-2H-pyran	131766-73-9	
Turpentine oil	8006-64-2	X
[3R-(3α,3β,7β,8α)]-1-(2,3,4,7,8,8a-Hexahydro-3,6,8,8-tetramethyl-1H-3a,7-methanoazulen-5-yl)ethan-1-one (Acetylcedrene)	32388-55-9	
(E)-Anethole (trans-anethole)	4180-23-8	
4-Methoxybenzyl alcohol (Anise alcohol)	105-13-5	
Benzyl salicylate	118-58-1	
2-(4-tert-Butylbenzyl)propionaldehyde	80-54-6	

² Special concern in the SCCS opinion (SCCS, 2012) is defined as due to the high number of reported cases, (>100 cases).

Substances	CAS RN	Substances of special concern according to SCCS (2012) ²
(Butylphenyl methylpropional)		
d-p-Mentha-1(6),8-dien-2-one (Carvone) / l-p-Mentha-1(6),8-dien-2-one (Carvone) / (S)-2-Methyl-5-(1-methylvinyl)cyclohex-2-en-1-one (Carvone)	99-49-0 / 6485-40-1 / 2244-16-8	
Cinnamaldehyde	104-55-2	X
Cinnamyl alcohol	104-54-1	X
Citronellol / (R)-3,7-dimethyloct-6-en-1-ol / (-)-3,7-dimethyloct-6-en-1-ol	106-22-9 / 1117-61-9 / 7540-51-4	
Coumarin	91-64-5	X
1-(2,6,6-Trimethyl-3-cyclohexen-1-yl)-2-buten-1-one (delta-DAMASCONE)	57378-68-4	
Eugenol	97-53-0	X
Farnesol	4602-84-0	X
Geraniol	106-24-1	X
4-(4-Hydroxy-4-methylpentyl)cyclohex-3-enecarbaldehyde (Hydroxyisohexyl 3-cyclohexene carboxaldehyde, HICC)	31906-04-4 / 51414-25-6	X
7-Hydroxycitronellal	107-75-5	X
Isoeugenol	97-54-1	X
Limonene	138-86-3	X
Methyl oct-2-ynoate	111-12-6	
Pin-2(3)-ene / Pin-2(10)-ene	80-56-8 / 127-91-3	
p-Mentha-1,4(8)-diene (Terpinolene)	586-62-9	
Ylang ylang ext. / Ylang ylang oil	83863-30-3 / 8006-81-3	X
Cinnamomum cassia leaf oil / Cinnamomum zeylanicum, ext.	8007-80-5 / 84649-98-9	
Neroli Oil / Orange, sour, ext.	8016-38-4 / 72968-50-4	
Lemon, ext.	84929-31-7	
Orange, sweet, Valencia, ext. / Orange, sweet, ext.	97766-30-8 / 8028-48-6	
Clove leaf oil	8000-34-8	X
Evernia furfuracea, ext.	90028-67-4	X
Evernia prunastri, ext.	90028-68-5	X

Substances	CAS RN	Substances of special concern according to SCCS (2012) ²
Jasmine, <i>Jasminum grandiflorum</i> , ext. / Jasmine, <i>Jasminum officinale</i> , ext. / Extract Jasmine (oil), Jasmine, <i>Jasminum grandiflorum</i> , ext.	84776-64-7 / 90045-94-6 / 8022-96-6	X
Oils, peppermint (<i>Mentha piperita</i>) / Peppermint, ext.	8006-90-4 / 84082-70-2	
Spearmint, ext. (<i>Mentha spicata</i>)	84696-51-5	
Balsams, Peru (<i>Myroxylon pereirae</i>)	8007-00-9	X
Sandalwood, ext. / Sandalwood oil	84787-70-2 / 8006-87-9	X

The 42 substances selected for this project include 19 of the 20 substances considered by the SCCS to be of special concern (SCCS, 2012) and marked with an 'X' in the right column in the table above. The remaining of the 20 substances considered by the SCCS (2012) to be of special concern, linalool, was not selected for this project as a harmonised classification as Skin Sens Cat 1B recently has been endorsed by the EU Risk Assessment Committee (RAC).

The full list of substances for evaluation in Phase 1 as provided by the Danish EPA is presented in Appendix 2. The list includes information of CAS Registry Number (CAS RN), substance name, harmonised classification for skin sensitisation in Category 1, notified classification for skin sensitisation in Category 1 (including sub-category 1A or 1B), approximate percentage of notified classifications including a classification for skin sensitisation in Category 1, REACH registration status, and priority according to the criteria set up by the Danish EPA.

3. Data collection and evaluation

The project was divided in two phases:

- Phase 1: Screening of the available data for the 42 selected substances with the purpose of performing a preliminary assessment whether the data justify a classification as a skin sensitiser in sub-category 1A (strong sensitisers) according to the CLP criteria, i.e. an identification of possible sub-category 1A candidates.
- Phase 2: A more detailed assessment of the data for those substances identified in Phase 1 as possible sub-category 1A candidates.

Data were collected for all the selected 42 substances as described in section 3.1 and the retrieved data were evaluated as described in section 3.2.

3.1 Data collection

Data from the SCCS (2012) opinion have been collected for 39 of the 42 selected substances. The three remaining substances (butyl methacrylate, 2-methyl-4-phenylpentanol, and the mixture of: trans-4-acetoxy-4-methyl-2-propyl-tetrahydro-2H-pyran; cis-4-acetoxy-4-methyl-2-propyl-tetrahydro-2H-pyran) were not included in the SCCS (2012) opinion.

The SCCS (2012) opinion was considered as the primary source of data for these 39 substances up to year 2011 for the purpose of the screening in this project as a very comprehensive literature search was performed as part of this opinion.

A supplementary literature search in the open literature has been performed covering the period from January 2009 and until October 2015 for the 39 substances addressed in the SCCS (2012) opinion in order to ensure that potentially relevant studies published after the adoption of the SCCS (2012) opinion also are taken into account. For the remaining 3 substances not addressed in the SCCS (2012) opinion, a complete literature search has been performed.

Then relevant information regarding skin sensitisation has been retrieved by searching literature databases such as SciFinder, PubMed and Scopus, as well as by searching IPCS INCHEM and Google for relevant international / national assessments and reports.

For the substances registered under REACH (27 substances, 28 CAS RN), the REACH registrations in the publicly accessible part of the REACH Registration Dossier Database, hosted on the ECHA website, were checked in order to identify eventual additional relevant information regarding skin sensitisation.

Additional information from the National Allergy Research Centre has been included if not already located from the above-mentioned sources.

The LOUS survey report (DK EPA, 2015) was also checked for relevant information; however, no relevant information for the purpose of the screening in this project was located.

The relevant information for the purpose of the screening in this project, i.e. for classification for skin sensitisation, including a possible classification in sub-category 1A, is human data and animal data (local lymph node assay, guinea pig maximization test and the Buehler assay). *In vitro* data and non-test data are also included in Phase 2 if retrieved.

3.1.1 Human data

Human evidence for classification of a substance for its skin sensitising potential can be based on positive data from patch testing, epidemiological studies showing allergic contact dermatitis caused by the substance, positive data from experimental studies in man and/or well documented episodes of allergic contact dermatitis, using a weight of evidence approach.

3.1.1.1 Epidemiological studies

The subjects examined are eczema patients, selected occupational groups, other selected groups, or general population, and the endpoint studied is elicitation. Large general population studies are scarce. Focused studies in selected populations are more common and provide insights on frequency of sensitisation compared to exposure.

3.1.1.2 Studies based on diagnostic patch testing

Diagnostic patch testing is conducted in order to diagnose allergic contact dermatitis (ACD) to a substance and is performed according to international standards by dermatologists. The subjects examined are eczema patients attending dermatology clinics and the endpoint studied is elicitation (as an indicator of previous sensitisation). Studies of diagnostic patch testing is usually reported as positive patch test frequencies, e.g. number of patients having a positive patch test result in relation to the total number of patients tested, as well as the percentage of positives. It is important to note how patients or individuals have been selected for patch testing; if all patients at a clinic with suspected ACD are patch tested they are often called consecutive or unselected patients at the clinic. Sometimes more aimed patch testing is performed among selected patients from a certain work environment or where exposure to certain groups of allergens, such as preservatives, fragrances or pigments, is suspected. Patch testing in selected patients usually results in higher frequencies of positive patch tests compared to tests performed in consecutive or unselected patients. This is to be considered under the evaluation of the results.

3.1.1.3 Case reports

The subjects examined are eczema patients diagnosed with contact allergy to a particular substance and the endpoint studied is elicitation. Individual cases are reported and are often the first reports made. Usually there are more details than in larger data-sets. They are useful in early detection of skin sensitisers and classification.

3.1.1.4 Experimental dose-response elicitation studies

This type of studies includes serial dilution patch tests or repeated open application tests (ROAT). The subjects examined are sensitised individuals (usually from diagnostic patch tests) and the endpoint studied is elicitation. Several protocols exist. This type of study provides an indication of the degree of sensitivity and of safe limits of exposure for induction as well as elicitation.

3.1.1.5 Experimental induction tests

This type of studies includes the Human Repeated Insult Patch Test (HRIPT) and the Human Maximization Test (HMT). The subjects examined are healthy volunteers and the endpoint studied is induction of sensitisation. For ethical reasons, such studies are no longer to be performed for EU regulations, including the CLP Regulation, but historical data may exist.

3.1.2 Animal data

There are three common animal test methods used to evaluate the potential of a substance to cause skin sensitisation:

- The mouse local lymph node assay (LLNA)
- The guinea pig maximisation test (GPMT)
- The Buehler assay

3.1.2.1 Mouse Local Lymph Node Assay

The mouse local lymph node assay (LLNA) (OECD TG 429) is used both for determination of skin sensitising potential (hazard identification) and for determination of relative skin sensitisation potency (hazard characterisation). In both instances the metric is cellular proliferation induced in the draining lymph nodes following topical exposure to a chemical, lymph node cell proliferation being causally and quantitatively correlated with the acquisition of skin sensitisation.

The test is considered positive when one of the doses results in a stimulation index (SI) ≥ 3 . Potency is measured as a function of derived EC3-values. The EC3-value is the amount of test chemical (% concentration, molar value or dose per unit area) calculated from the dose-response data to elicit a stimulation index of 3. An inverse relationship exists between EC3-value and potency meaning that extremely potent sensitisers have extremely low EC3-values.

It is known that the choice of vehicle may provide a variable EC3 value, which may significantly influence the skin sensitising potency and make it difficult to categorise/subcategorise the substance.

Different variants of the LLNA exist, namely the reduced LLNA (rLLNA) which has been added as an option in the amended OECD TG 429 in 2010, the LLNA: DA (OECD TG 442A), and the LLNA: BrdU-ELISA (OECD TG 442B).

The rLLNA uses only a negative control group and the equivalent of the high-dose group from the full LLNA. The rLLNA does not allow the determination of the potency of a sensitising chemical as only one dose is tested. The rLLNA also uses fewer animals than the full LLNA and should only be used in those circumstances where dose-response information are not required (e.g. to confirm a negative prediction of skin sensitising potential) and thus should not be used for sub-categorisation of skin sensitisers.

The test is considered positive in the LLNA: DA when the stimulation index is ≥ 1.8 and in the LLNA: BrdU-ELISA when the stimulation index is ≥ 1.6 . There is no guidance on how the LLNA: DA or the LLNA: BrdU-ELISA can be used for sub-categorisation.

3.1.2.2 Guinea Pig Maximisation Test

The guinea pig maximisation test (GPMT) (OECD TG 406) has been used for over 40 years to detect the skin sensitising potential of chemicals through a test system maximizing the sensitivity by both intradermal and epidermal induction and use of an adjuvant (Freund's Complete Adjuvant). The intradermal induction is made by injection. Consequently the test is not suited for substances which cannot be made up into a liquid formulation.

The GPMT was originally designed to maximise the ability to identify a sensitisation hazard, rather than to determine skin sensitisation potency. Yet, potency categorisation is possible on the basis of the concentration of test material used for intradermal induction and the percentage of guinea pigs sensitised. However, it should be recognised that there is often a degree of uncertainty associated with the derivation of allergenic potencies from the GPMT.

3.1.2.3 Buehler assay

The Buehler assay (OECD TG 406) has been in use for the last 40 years to detect the skin sensitising potential of chemicals using epidermal occluded exposure. The skin barrier of the test species (guinea pig) is kept intact in this assay.

The Buehler test was originally designed to identify a sensitisation hazard, rather than to determine skin sensitisation potency. Yet, potency can be categorised using the results of the Buehler assay on the basis of the number of animals sensitised and the concentration of the test material used for the epidermal induction. However, it should be recognised that there is often a degree of uncertainty associated with the derivation of allergenic potencies from the Buehler assay.

3.1.2.4 Non-compliant skin sensitisation tests

For the 42 selected substances, several older animal studies have been retrieved from the various literature sources. Many of these studies have not been performed according to the present internationally accepted test guidelines.

In vivo test methods which do not comply with recognised test guidelines are strongly discouraged in the CLP Guidance for the identification of skin sensitisers or assessment of skin sensitising potency. The results of such tests have to be well-validated with scientific justification and evaluated carefully, but may provide supportive evidence. If doubts exist about the validity and the interpretation of the results, the evaluation needs to be taken by using a weight of evidence approach.

The results of such older animal studies have been included in both the Phase 1 and Phase 2 evaluations for transparency reasons, but only as supplementary evidence.

3.1.3 *In vitro* data

Two *in vitro* skin sensitisation methods have recently (February 2015) been adopted as OECD test guidelines.

One test is the “*in chemico*” skin sensitisation ‘Direct Peptide Reactivity Assay’ (DPRA) (OECD TG 442C). This method measures the ability of chemicals to react with proteins (haptentation), a determinant step in the induction of skin sensitisation. It is based on the chemical reactivity of the compound under investigation, with lysine and cysteine residues. The test method, however, is not proposed as a stand-alone full replacement test for the *in vivo* animal studies since the DPRA test is covering only one single biological step in the skin sensitisation pathway and does not consider metabolic capacity. DPRA information may also have the potential to contribute to potency assessment.

Another test is the *in vitro* skin sensitisation ‘ARE-Nrf2 luciferase test’ (OECD TG 442D). This method measures activation of keratinocytes and determines the direct reactivity of sensitising material to key cysteine residues of Keap1, a regulator of Nrf2. The Nrf2-Keap1-ARE regulatory pathway is considered one of the most relevant pathways for the identification of potential skin sensitisers. The test method, however, is not proposed as a stand-alone full replacement test for the *in vivo* animal studies since it addresses only one single biological step in the overall mechanism of skin sensitisation. Considering the known limitations of this test such as the limited consideration of metabolic aspects and the ability to detect only cysteine-reactive chemicals, it has been recommended that the method should only be used in combination with other information sources.

In vitro data are currently not part of the classification criteria for skin sensitisers according to the CLP Regulation and Guidance.

3.1.4 Non-testing data

At present no formally validated non-testing systems exist to predict skin sensitising potential. However, data such as structural alert data or data to show that the chemical structure of a molecule is similar to that of known sensitisers (e.g. QSARs or expert systems) may form part of the weight of evidence for classification.

3.2 Evaluation of data

Phase 1 consisted of a screening of the available data for the selected 42 substances with the purpose of performing a preliminary assessment if the data could be sufficient for a classification in sub-category 1A according to the CLP criteria, i.e. an identification of possible sub-category 1A candidates. Therefore, a thorough evaluation of the retrieved data was not performed in Phase 1.

In Phase 2, the quality of the data for the selected substances was assessed as described in section 3.2.1 and the data considered valid for the purpose of the screening in this project were then assessed against the CLP classification criteria for skin sensitisation with special focus on whether classification in sub-category 1A is justified. The CLP classification criteria are summarised in section 3.2.2.

3.2.1 Quality of data

For each substance taken forward to Phase 2 the quality of the relevant data was assessed according to the Klimisch criteria (Klimisch et al., 1997).

Each reference was given a score 1-4:

- 1 = reliable without restrictions: “studies or data generated according to generally valid and/or internationally accepted testing guidelines (preferably performed according to GLP) or in which the test parameters documented are based on a specific (national) testing guideline or in which all parameters described are closely related/comparable to a guideline method.”
- 2 = reliable with restrictions: “studies or data (mostly not performed according to GLP), in which the test parameters documented do not totally comply with the specific testing guideline, but are sufficient to accept the data or in which investigations are described which cannot be subsumed under a testing guideline, but which are nevertheless well documented and scientifically acceptable.”
- 3 = not reliable: “studies or data in which there were interferences between the measuring system and the test substance or in which organisms/test systems were used which are not relevant in relation to the exposure (e.g. un-physiological pathways of application) or which were carried out or generated according to a method which is not acceptable, the documentation of which is not sufficient for assessment and which is not convincing for an expert judgment.”
- 4 = not assignable: “studies or data which do not give sufficient experimental details and which are only listed in short abstracts or secondary literature (books, reviews, etc.).”

Then, ‘reliable’ data (assigned score 1 or 2) were then assessed against the CLP classification criteria for skin sensitisation with special focus on whether classification in sub-category 1A is justified.

Other data (assigned score 4) were also included in the assessment.

Not ‘reliable’ data (assigned score 3) were not included in the assessment.

3.3 Comparison with classification criteria according to the CLP Regulation

The available data for the substances evaluated in Phase 1 and Phase 2 were compared with the criteria for classification as skin sensitisers in sub-category 1A according to the CLP Regulation (EC no. 1272/2008). The classification criteria are presented below. The criteria are complex and further guidance for the use of the criteria and on how to evaluate the data are found in the CLP Guidance document “Guidance on the application of the CLP criteria” which is available from the European Chemicals Agency website (ECHA, 2015).

3.3.1 Classification criteria for sub-category 1A

According to Annex I, section 3.4.2.2.1.3 in the CLP regulation (EC no. 1272/2008): “*Effects seen in either humans or animals will normally justify classification in a weight of evidence approach for skin sensitisers as described in section 3.4.2.2.2. Substances may be allocated to one of the two sub-categories 1A or 1B using a weight of evidence approach in accordance with the criteria given in Table 3.4.2 and on the basis of reliable and good quality evidence from human cases or epidemiological studies and/or observations from appropriate studies in experimental animals according to the guidance values provided in sections 3.4.2.2.2.1 and 3.4.2.2.3.2 for sub-category 1A and in sections 3.4.2.2.2.2 and 3.4.2.2.3.3 for sub-category 1B.*”.

3.3.1.1 Criteria given in Table 3.4.2 for sub-category 1A

Substances showing a high frequency of occurrence in humans and/or a high potency in animals can be presumed to have the potential to produce significant sensitisation in humans. Severity of reaction may also be considered.

When considering human evidence, it is necessary to take into account the size of the population exposed and the extent of exposure and frequency, and thus the consideration is on a case by case basis. Human data should be incorporated with animal data to decide the sub-categorisation.

3.3.1.2 Criteria for sub-category 1A listed in CLP Annex I, 3.4.2.2.2.1 – human data:

Human evidence for sub-category 1A can include:

- Positive responses at $\leq 500 \mu\text{g}/\text{cm}^2$ (HRIPT, HMT – induction threshold);
- Diagnostic patch test data where there is a relatively high and substantial incidence of reactions in a defined population in relation to relatively low exposure;
- Other epidemiological evidence where there is a relatively high and substantial incidence of allergic contact dermatitis in relation to relatively low exposure.

High and substantial incidence of reactions:

For human diagnostic patch test data, only one or two types of the following information regarding a relative high and substantial incidence of reactions in a defined population may be sufficient for sub-categorisation:

Human diagnostic patch test data	High frequency	Low / moderate frequency
General population studies	≥ 0.2 %	< 0.2 %
Dermatitis patients (unselected, consecutive)	≥ 1.0 %	< 1.0 %
Selected dermatitis patients (aimed testing, usually special test series)	≥ 2.0 %	< 2.0 %
Work place studies:		
1: all or randomly selected workers	≥ 0.4 %	< 0.4 %
2: selected workers with known exposure or dermatitis	≥ 1.0 %	< 1.0 %
Number of published cases	≥ 100 cases	< 100 cases

Relatively low exposure:

Relatively high or low exposure relates to the concentrations people are exposed to in their daily lives, in the workplace, or other conditions and resulting in sensitisation. The exposure index is the sum of the scores obtained from the information in each row of the table below, i.e. a response in each row is necessary. A relatively low exposure is indicated if the exposure index is between 1 and 4.

Exposure data	Relatively low exposure	Relatively high exposure
Concentration / dose	< 1.0 % < 500 µg/cm ² (score 0)	≥ 1.0 % ≥ 500 µg/cm ² (score 2)
Repeated exposure	< once/daily (score 1)	≥ once/daily (score 2)
Number of exposures (irrespective of concentration of sensitiser)	< 100 exposures (score 0)	≥ 100 exposures (score 2)

3.3.1.3 Criteria for sub-category 1A listed in CLP Annex I, 3.4.2.2.3.2 – non-human data:

Animal test results for sub-category 1A:

- Local lymph node assay: EC₃ value ≤ 2 %
- Guinea pig maximisation test: ≥ 30 % responding at ≤ 0,1 % intradermal induction dose or ≥ 60 % responding at > 0,1 % to ≤ 1 % intradermal induction dose
- Buehler assay: ≥ 15 % responding at ≤ 0,2 % topical induction dose or ≥ 60 % responding at > 0,2 % to ≤ 20 % topical induction dose

3.3.1.4 Weight of evidence

For classification of a substance, evidence shall include any or all of the following using a weight of evidence approach:

- Positive data from patch testing, normally obtained in more than one dermatology clinic
- Epidemiological studies showing allergic contact dermatitis caused by the substance
- Positive data from appropriate animal studies
- Positive data from experimental studies in man
- Well documented episodes of allergic contact dermatitis, normally obtained in more than one dermatology clinic
- Severity of reaction may also be considered

Positive effects seen in either humans or animals for skin sensitisation will normally justify classification. In cases where evidence is available from both sources, and there is conflict between the results, the quality and reliability of the evidence from both sources must be assessed in order to decide on the classification on a case-by-case basis.

4. Results, Phase 1

Phase 1 consisted of a screening of the available data for the selected 42 substances with the purpose of performing a preliminary assessment if the available data could be sufficient for a classification in sub-category 1A according to the CLP criteria, i.e. an identification of possible sub-category 1A candidates.

Based on this preliminary assessment, each of the 42 selected substances was given a priority 1 or 2:

- Priority 1 was given to those substances which were considered as possible sub-category 1A candidates, in total 20 substances.
- Priority 2 was given to those substances which were not considered as possible sub-category 1A candidates, in total 22 substances.

The justification for the prioritisation of the substances as 1 or 2 is presented in Appendix 3.

The 20 substances given a priority 1 are listed in the table below:

Substances	CAS RN	Substances of special concern according to SCCS (2012) ³
Butyl methacrylate	97-88-1	
Citral	5392-40-5	X
Turpentine oil	8006-64-2	X
Cinnamaldehyde	104-55-2	X
Cinnamyl alcohol	104-54-1	X
Coumarin	91-64-5	X
Eugenol	97-53-0	X
Farnesol	4602-84-0	X
Geraniol	106-24-1	X
7-Hydroxycitronellal	107-75-5	X
Limonene	138-86-3	X
Methyl oct-2-ynoate	111-12-6	
Ylang ylang ext. / Ylang ylang oil	83863-30-3 / 8006-81-3	X
<i>Cinnamomum cassia</i> leaf oil / <i>Cinnamomum zeylanicum</i> , ext.	8007-80-5 / 84649-98-9	

³ Special concern in the SCCS opinion (SCCS, 2012) is defined as due to the high number of reported cases, (>100 cases).

Substances	CAS RN	Substances of special concern according to SCCS (2012) ³
Clove leaf oil	8000-34-8	X
<i>Evernia furfuracea</i> , ext.	90028-67-4	X
<i>Evernia prunastri</i> , ext.	90028-68-5	X
Jasmine, <i>Jasminum grandiflorum</i> , ext. / Jasmine, <i>Jasminum officinale</i> , ext. / Extract Jasmine (oil), Jasmine, <i>Jasminum grandiflorum</i> , ext.	84776-64-7 / 90045-94-6 / 8022-96-6	X
Balsams, Peru (<i>Myroxylon pereirae</i>)	8007-00-9	X
Sandalwood, ext. / Sandalwood oil	84787-70-2 / 8006-87-9	X

Among these 20 substances, five were identified in the LOUS report as having a harmonised classification for skin sensitisation in Category 1 (H317) according to the CLP criteria. These substances are: Butyl methacrylate, citral, d-limonene, l-limonene, and turpentine oil.

Among these 20 substances, 17 substances were identified in the SCCS (2012) opinion as established contact allergens in humans and considered to be of special concern as they have given rise to at least 100 reported cases (the substances marked having assigned an 'X' in the right column in the table above).

The remaining three substances considered to be of special concern in the SCCS (2012) opinion (hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), isoeugenol and linalool) were not prioritised further in this project. HICC and isoeugenol were among the 42 substances originally selected for the Phase 1 preliminary assessment. These two substances were, however, not given a priority 1 in the Phase 1 assessment as a harmonised classification in sub-category 1A already has been proposed, i.e. the purpose of the project has already been fulfilled. Linalool was not selected for this project as a harmonised classification in sub-category 1B recently has been endorsed by the EU Risk Assessment Committee (RAC).

Among the 20 identified possible sub-category 1A candidates, a further prioritisation was performed for the selection of the Phase 2 substances, i.e. the substances to go through the more detailed assessment of the relevant data.

The highest priority was given to the substances identified in the SCCS (2012) opinion as established contact allergens in humans and considered to be of special concern (marked with an 'X' in the right column of the table) and/or based on the clinical experience from the National Allergy Research Centre.

The 11 substances taken forward to Phase 2 are listed in the table below:

Substances	CAS RN	Substances of special concern according to SCCS (2012)
Citral	5392-40-5	X
Cinnamaldehyde	104-55-2	X
Cinnamyl alcohol	104-54-1	X
Coumarin	91-64-5	X
Eugenol	97-53-0	X
Farnesol	4602-84-0	X
Geraniol	106-24-1	X
7-Hydroxycitronellal	107-75-5	X
Methyl oct-2-ynoate	111-12-6	
<i>Cinnamomum cassia</i> leaf oil / <i>Cinnamomum zeylanicum</i>, ext.	8007-80-5 / 84649-98-9	
<i>Evernia prunastri</i>, ext.	90028-68-5	X

5. Results, Phase 2

This chapter presents the results of the detailed assessment performed for the 11 substances selected for Phase 2. The results are presented as a summary of the available data, comparison with CLP Regulation criteria for classification in sub-category 1A, and conclusion on classification for each substance. Appendices 4-14 present the full overview of the available studies/data for the substances and the evaluation of their skin sensitising potential.

Most of the studies included in the 11 substance evaluations have been cited from secondary literature, i.e. SCCS/SCCNFP opinions, REACH-RD and reviews and therefore assigned reliability score 4 “Not assignable” according to the Klimisch criteria (Klimisch et al., 1997) as described above in Section 3.2.1. The remaining part of the studies included in the 11 substance evaluations have been available in form of publications from the open literature and therefore assigned reliability score 2 “Reliable with restrictions” according to the Klimisch criteria. A substantial part of those studies cited from secondary literature are unpublished data from the Industry.

The studies have been assessed against the CLP classification criteria for skin sensitisation with special focus on whether classification in sub-category 1A is justified.

One of the elements in the criteria for classification in sub-category 1A based on human patch test data is ‘relatively low exposure’. Relatively high or low exposure relates to the concentrations of the substances in cosmetics and other consumer products which individuals are exposed to in their daily lives, in the workplace, or other conditions and resulting in sensitisation. A cut-off concentration of 1 % has been set in order to discriminate between relatively high exposure (≥ 1.0 %) and relatively low exposure (< 1.0 %).

In the SCCS opinion on fragrance allergens in cosmetic products (SCCS, 2012) it is mentioned that based on data from the fragrance industry (International Fragrance Association, IFRA), 80 % of the total fragrance chemical volume is used in cosmetics and 20 % in household products such as e.g., detergents. However, no quantitative information on the concentrations of fragrances in cosmetics and other consumer products is available in the SCCS opinion. Whether the exposure for the 11 Phase 2 substances is relatively high or relatively low from use as fragrance in cosmetics and in other consumer products has therefore been evaluated based on the IFRA standard limit of each substance in each of 11 finished product categories (IFRA, 2015), i.e., if the IFRA standard limit is < 1.0 % the exposure is thus considered as being relatively low. The IFRA standard limits have generally been set based on quantitative risk assessment (QRA).

For the application of QRA, consumer product types were grouped according to key parameters identified within the QRA approach, i.e., sensitisation assessment factors (SAFs) and consumer product exposure. By using these parameters, 11 different IFRA QRA categories for dermal sensitisation were specified by the QRA Expert Group. For many categories there is generally a wide diversity of product types including cosmetics as well as other consumer products. This is because the categories are based on SAFs and consumer product exposure, not on the functional similarity of each product type.

The overall ‘category consumer exposure level’ is driven by the product type in that category with the combined highest consumer exposure level and highest SAF. These data are used with the WoE NESIL (Weight of Evidence No Expected Sensitization Induction Level) to calculate the ‘acceptable exposure level’ (AEL) for individual fragrance ingredients (AEL is the NESIL divided by the SAF and multiplied by the consumer exposure level).

A default maximum level of the fragrance ingredients identified as dermal sensitisers has been set for practical considerations. This 'maximum pragmatic level' is defined as the level not exceeding the usual concentration of the fragrance in the finished product. If the AEL derived from QRA is less than the 'maximum pragmatic level', the AEL is applied as the IFRA standard limit. Otherwise, the 'maximum pragmatic level' is applied as the IFRA standard limit.

The tables in the individual substance evaluations (Appendices 4-14) present the IFRA standard limit for each of the 11 IFRA QRA categories, as well as the product type that drives the 'category consumer exposure level'.

5.1 Citral

5.1.1 Summary of the available data

5.1.1.1 Human data

A total of 30 results from patch test population studies, 7 HRIPTs, 14 HMTs and 2 case studies were identified for citral. The positive patch test frequencies from all of the reported patch test population studies vary between 0.3 and 16.7% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0.3 and 4.8% (8 studies) and in studies with selected dermatitis patients positive reactions range between 0.3 and 16.7% (22 studies). The total number of published cases is > 300. Sensitisation was reported in 3/7 HRIPT studies after exposure to 3876 µg/cm² (5%) and in 13/14 HMT studies after exposure to 1379 µg/cm².

5.1.1.2 Non-human information

A total of 12 LLNAs, 6 GPMTs and 1 Buehler test were identified testing skin sensitising effects of citral. The reported EC3 values for citral ranged between 1.2% and 15% in different vehicles. In the GPMTs sensitisation was observed but not quantified (i.e. number of animals affected) in 3/6 studies with intradermal induction doses of 0.4, 5 and 10% citral. In the other GPMTs sensitisation was observed in 60% of the animals after an intradermal induction dose of 0.1% and in 60-100% of the animals after an intradermal induction dose of 25% citral. Sensitisation was also observed but not quantified (i.e. number of animals affected) in the Buehler test with an induction concentration of 20% citral.

No relevant *in vitro* studies on citral (i.e. OECD TG 442C and OECD TG 442D) were identified in the literature.

5.1.1.3 Exposure

According to data from IFRA (2013a) the exposure of citral when used as fragrance in cosmetics and in other consumer products appears to be low.

5.1.2 Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 0.3 and 4.8% with 3/8 studies reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 0.3 and 16.7% with 14 out of 22 studies reporting frequencies higher than 2%. In addition to this there are more than 300 published cases of positive patch test reactions to citral. According to the CLP criteria a frequency ≥ 1% for unselected/consecutive dermatitis patients and/or ≥ 2% for selected dermatitis patients and/or a total number of published cases ≥ 100, equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that citral causes a high frequency of occurrence of skin sensitisation based on patch test data from a minority of unselected dermatitis patient studies and a majority of selected dermatitis patient studies and the number of published cases.

In regard to the HRIPT/HMT data the positive response reported at > 500 µg/cm² for HRIPT/HMT induction threshold indicate evidence for sub-category 1B according to Annex I: 3.4.2.2.2.2.

In the 14 LLNAs EC3 values between 1.2 and 15% were reported for citral. Two out of the 14 LLNAs reported an EC3 value <2%. According to the CLP Regulation an EC3 value ≤ 2% indicates classification of a substance in sub-category 1A whereas an EC3 value > 2% indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.). Thus, these two studies indicate classification of citral in sub-category 1A. However, in the other 12 LLNAs, the EC3 value was >2% with only one of these 12 LLNAs reporting an EC3 value (2.1%) borderline to the cut-off criteria for classification in sub-category 1A or 1B indicating classification of citral in sub-category 1B. Based on a weight of evidence for the LLNAs, classification of citral in sub-category 1B seems justified.

In 1/6 of the GPMTs sensitisation was observed in 60% of the animals after an intradermal induction dose of 0.1%. According to the CLP criteria a positive response ≥ 30% of the animals responding at ≤ 0.1% intradermal induction dose indicates classification of a substance in sub-category 1A (Annex I: 3.4.2.2.3.2.) and thus, this study indicates classification of citral into sub-category 1A. In 2/6 GPMTs sensitisation was observed in 60-100% of the animals after an intradermal induction dose of 25% citral. According to the CLP criteria a positive response ≥ 30% responding at > 1% intradermal induction dose indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.); thus, these two studies indicate classification of citral in sub-category 1B. In 3/6 the GPMTs sensitisation was reported to be observed but not quantified (i.e. number of animals affected) with intradermal induction doses of 0.4, 5 and 10% citral; therefore, these GPMTs cannot be compared with the classification criteria.

Sensitisation was also reported to be observed but not quantified (i.e. number of animals affected) in the Buehler test with an induction concentration of 20% citral; therefore, this study cannot be compared with the classification criteria.

Overall, there is clear evidence for classification in sub-category 1A based on the human patch test studies showing a high frequency of occurrence of skin sensitisation and the total number of cases combined with the estimated low exposure. The results from the animal studies are equivocal, mainly indicating classification in sub-category 1B. A classification as a skin sensitizer in sub-category 1A is thus warranted for citral.

5.1.3 Conclusions on classification and labelling

Based on the high frequency of sensitisation observed in human patch test studies and the high number of published cases combined with the estimated low exposure, a classification of citral as a skin sensitizer in sub-category 1A is justified.

5.2 Cinnamaldehyde

5.2.1 Summary of the available data

5.2.1.1 Human data

A total of 52 results from patch test population studies, 7 HRIPTs, 14 HMTs and 2 case studies were identified with cinnamaldehyde. The positive patch test frequencies from all of the reported patch test population studies vary between 0.93 and 90% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0.93 and 32.5% (8 studies) and in studies with selected dermatitis patients positive reactions range between 1.2 and 90% (44 studies). A single study in workers reported positive patch test reactions in 1%. The total number of published cases is > 2300. A LOEL-HRIPT/HMT (induction) of 775 µg/cm² was established for cinnamaldehyde by the RIFM Expert Panel based on unpublished reports.

5.2.1.2 Non-human information

A total of 22 LLNAs and 2 LLNA BrdU-ELISA tests were identified testing skin sensitising effects of cinnamaldehyde. The reported EC3 values for cinnamaldehyde ranged between 0.2% and 3.1% in different vehicles.

No relevant *in vitro* studies on cinnamaldehyde (i.e. OECD TG 442C and OECD TG 442D) were identified in the literature.

5.2.1.3 Exposure

According to data from IFRA (2013b) the exposure of cinnamaldehyde when used as fragrance in cosmetics and in other consumer products appears to be low.

5.2.2 Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 0.93 and 32.5% with 7/8 studies reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 1.2 and 90% with 37 out of 44 studies reporting frequencies higher than 2%. A single study in workers reported positive patch test reactions in 1%. In addition to this there are more than 2300 published cases of positive patch test reactions to cinnamaldehyde. According to the CLP criteria a frequency ≥ 1% for unselected/consecutive dermatitis patients and/or ≥ 2% for selected dermatitis patients and/or ≥ 1% for selected workers with known exposure or dermatitis and/or a total number of published cases ≥ 100, equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that cinnamaldehyde causes a *high frequency* of occurrence of skin sensitisation based on these four types of information.

In regard to the HRIPT/HMT data the positive response reported at > 500 µg/cm² for HRIPT/HMT induction threshold indicate evidence for sub-category 1B according to Annex I: 3.4.2.2.2.2.

In the 22 LLNAs EC3 values between 0.2 and 3.1% were reported for cinnamaldehyde with 21/22 EC3 values <2%. According to the CLP Regulation an EC3 value ≤ 2% indicates classification of a substance in sub-category 1A (Annex I: 3.4.2.2.3.2.). Hence, data from LLNAs indicate classification of cinnamaldehyde in sub-category 1A.

Overall, there is clear evidence for classification in sub-category 1A based on the human patch test studies showing a high frequency of occurrence of skin sensitisation and the total number of cases combined with the estimated low exposure. Data from HRIPT/HMT indicate evidence for sub-category 1B. Data from the LLNAs support a sub-category 1A classification. A classification as a skin sensitiser in sub-category 1A is thus warranted for cinnamaldehyde.

5.2.3 Conclusions on classification and labelling

Based on the high frequency of sensitisation observed in human patch test studies and the high number of published cases combined with the estimated low exposure and supported by data from LLNAs, a classification of cinnamaldehyde as a skin sensitiser in sub-category 1A is justified.

5.3 Cinnamyl alcohol

5.3.1 Summary of the available data

5.3.1.1 Human data

A total of 34 patch test population studies, 3 HRIPTs, 25 HMTs and 4 case studies, were identified with cinnamyl alcohol. The positive patch test frequencies from all of the reported patch test studies vary between 0.56 and 100% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0.56 and 1.8% (4 studies) and in studies with selected dermatitis patients positive reactions range between 1.5 and 100% (30 studies). The total number of published cases is > 600. A LOEL (induction) of 4724 µg/cm² was derived from the HRIPT/HMT studies.

5.3.1.2 Non-human information

A total of 5 LLNAs (two of which were reported to be conducted in accordance with OECD TG 429), 2 GPMT, 1 Freund's complete adjuvant test (FCAT) and 1 Buehler test were identified testing the skin sensitisation of cinnamyl alcohol. EC3 values between 17.9 and 30% were reported for cinnamyl alcohol in the LLNAs and positive reactions were observed in a GPMT (30% positive) and FCAT (15% positive) at intradermal induction doses of 25 and 100%, respectively. No positive reactions were observed in the Buehler test.

No relevant *in vitro* studies on cinnamyl alcohol (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

5.3.1.3 Exposure

According to data from IFRA (2008a) the exposure of cinnamyl alcohol when used as fragrance in cosmetics and in other consumer products appears to be low.

5.3.2 Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 0.56 and 1.8% with 1/4 studies reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 1.5 and 100% with 28 out of 30 studies reporting frequencies higher than 2%. In addition to this there are more than 600 published cases of positive patch test reactions to cinnamyl alcohol. According to the CLP criteria a frequency ≥ 1% for unselected/consecutive dermatitis patients and/or ≥ 2% for selected dermatitis patients and/or a total number of published cases ≥ 100, equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that cinnamyl alcohol causes a high frequency of occurrence of skin sensitisation based on frequencies in selected dermatitis patients and total number of cases.

In regard to the HRIPT/HMT data the positive response reported at > 500 µg/cm² for HRIPT/HMT induction threshold indicate evidence for sub-category 1B according to Annex I: 3.4.2.2.2.2.

In the LLNAs EC3 values between 17.9 and 30% were reported for cinnamyl alcohol. According to the CLP Regulation an EC3 value larger than 2% indicates placement of cinnamyl alcohol into sub-category 1B (Annex I: 3.4.2.2.3.2.).

In the GPMT sensitisation was observed in 30% of the animals after an intradermal induction dose of 25% cinnamyl alcohol. According to the CLP criteria a positive response ≥ 30% of the animals responding at >1% intradermal induction dose indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.).

No sensitisation was observed in the Buehler test after an induction dose of 30% cinnamyl alcohol.

Overall, there is clear evidence for classification in sub-category 1A based on the frequency of sensitisation in human patch test studies with selected dermatitis patients and the total number of cases combined with the estimated low exposure. Data from HRIPT/HMT indicate evidence for sub-category 1B. All animal studies indicate a classification in sub-category 1B. A classification as a skin sensitiser in sub-category 1A is warranted for cinnamyl alcohol.

5.3.3 Conclusions on classification and labelling

Based on the high frequency of sensitisation observed in human patch test studies and the high number of published cases combined with the estimated low exposure a classification of cinnamyl alcohol as a skin sensitiser in sub-category 1A is justified.

5.4 Coumarin

5.4.1 Summary of the available data

5.4.1.1 Human data

A total of 25 patch test population studies and 2 case studies, one of which included a ROAT, were identified with coumarin. The positive patch test frequencies from all of the reported patch test studies vary between 0 and 10% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0 and 0.8% (7 studies) and in studies with selected dermatitis patients positive reactions range between 0 and 10% (19 studies). The total number of published cases is > 200. A LOEL (induction) of 8858 µg/cm² was derived from the HRIPT/HMT studies.

5.4.1.2 Non-human information

A total of 20 LLNAs and 1 GPMT were identified testing skin sensitising effects of coumarin. The collected evidence from the LLNAs indicates an EC₃ for coumarin of ca. 50%. Sensitisation was not observed in the GPMT after an intradermal induction dose of 0.5% coumarin.

Contaminants in coumarin may act as weak or moderate sensitizers (SCCS, 2012).

No relevant *in vitro* studies on coumarin (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

5.4.1.3 Exposure

According to data from IFRA (2008b) the exposure of coumarin when used as fragrance in cosmetics and in other consumer products appears to be low.

5.4.2 Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 0 and 0.8% i.e. all 7 studies reporting frequencies lower than 1%. For selected dermatitis patients positive reactions range between 0 and 10% with 9 out of 19 studies reporting frequencies higher than 2%. In addition to this there are more than 200 published cases of positive patch test reactions to coumarin. According to the CLP criteria a frequency ≥ 1% for unselected/consecutive dermatitis patients and/or ≥ 2% for selected dermatitis patients and/or a total number of published cases ≥ 100, equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that coumarin causes a low/moderate frequency of occurrence of skin sensitisation based on unselected/consecutive dermatitis patients and 10/19 studies with selected dermatitis patients. The remaining studies with selected dermatitis patients (9/19) and number of published cases shows that coumarin causes a high frequency of occurrence of skin sensitisation in humans.

In regard to the HRIPT/HMT data the positive response reported at > 500 µg/cm² for HRIPT/HMT induction threshold indicate classification of coumarin in sub-category 1B according to Annex I: 3.4.2.2.2.2.

The collected evidence from the LLNAs indicates an EC₃ for coumarin of ca. 50%. According to the CLP Regulation an EC₃ value ≤ 2% indicates classification of a substance in sub-category 1A whereas an EC₃ value > 2% indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.). Thus, all studies indicate classification of coumarin in sub-category 1B.

The single GPMT with an intradermal induction dose of 0.5% gave no positive reactions which does not justify sub-categorisation (Table 3.4.3).

Overall, there is evidence for classification in sub-category 1A based on the number of cases combined with the estimated low exposure and supported by patch test data from selected dermatitis patients. Data from HRIPT/HMT indicate evidence for sub-category 1B. Data from LLNAs indicate a classification in sub-category 1B. A classification as a skin sensitiser in sub-category 1A is warranted for coumarin.

5.4.3 Conclusions on classification and labelling

Based on the number of cases combined with the estimated low exposure and supported by patch test data from selected dermatitis patients, a classification of coumarin as a skin sensitiser in sub-category 1A is justified.

5.5 Eugenol

5.5.1 Summary of the available data

5.5.1.1 Human data

A total of 36 patch test population studies, 1 ROAT and 1 case study were identified with eugenol. The positive patch test frequencies from all of the reported patch test studies vary between 0.3 and 55.4% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0.3 and 1.9% (5 studies) and in studies with selected dermatitis patients positive reactions range between 0.62 and 55.4% (31 studies). The total number of published cases is > 700. A NESIL from HRIPT studies of 5900 µg/cm² was derived based on weight of evidence by the RIFM Expert Panel.

5.5.1.2 Non-human information

A total of 15 LLNAs and one GPMT were identified testing the skin sensitisation of eugenol. EC3 values were reported in 13 studies and ranged between 4.2 and 25.1%. Positive reactions (20%) were observed in the GPMT at an intradermal induction dose of 5%.

No relevant *in vitro* studies on eugenol (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

5.5.1.3 Exposure

According to data from IFRA (2008c) the exposure of eugenol when used as fragrance in cosmetics and in other consumer products appears to be low.

5.5.2 Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 0.3 and 1.9% with 2 out of 5 studies reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 0.62 and 55.4% with 25 out of 31 studies reporting frequencies higher than 2%. In addition to this there are more than 700 published cases of positive patch test reactions to eugenol. According to the CLP criteria a frequency ≥ 1% for unselected/consecutive dermatitis patients and/or ≥ 2% for selected dermatitis patients and/or a total number of published cases ≥ 100, equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that eugenol causes a high frequency of occurrence of skin sensitisation based on the frequency of positive reactions mainly in selected dermatitis patients (>2% in 21/27 studies) and the total number of cases.

In regard to the HRIPT/HMT data the positive response reported at > 500 µg/cm² for HRIPT/HMT induction threshold indicate evidence for sub-category 1B according to Annex I: 3.4.2.2.2.2.

In the LLNA tests, EC3 values between 4.2 and 25.1% (13 studies) were reported for eugenol. According to the CLP Regulation an EC3 value larger than 2 indicates placement of eugenol into sub-category 1B (Annex I: 3.4.2.2.3.2.).

In the GPMT sensitisation was observed in 20% of the animals after an intradermal induction dose of 5% which does not justify sub-categorisation into either sub-category 1A or 1B (Annex I: 3.4.2.2.3.2.).

Overall, there is clear evidence for classification in sub-category 1A based on the frequency of sensitisation in human patch test studies mainly with selected dermatitis patients and the total number of cases combined with the estimated low exposure. Data from HRIPT/HMT indicate evidence for sub-category 1B. Data from LLNAs indicate a classification in sub-category 1B. A classification as a skin sensitizer in sub-category 1A is warranted for eugenol.

5.5.3 Conclusions on classification and labelling

Based on the high frequency of sensitisation observed in human patch test studies and the high number of published cases, combined with the estimated low exposure, a classification of eugenol as a skin sensitizer in sub-category 1A is justified.

5.6 Farnesol

5.6.1 Summary of the available data

5.6.1.1 Human data

A total of 20 patch test population studies, 3 HRIPTs, 11 HMTs and 2 case studies were identified with farnesol. The positive patch test frequencies from all of the reported patch test population studies vary between 0.02 and 13.2% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0.35 and 0.9% (4 studies) and in studies with selected dermatitis patients positive reactions range between 0.02 and 13.2% (16 studies). The total number of published cases is > 250. Positive responses after farnesol were seen at concentrations $\geq 6900 \mu\text{g}/\text{cm}^2$ in 5/11 HMTs.

5.6.1.2 Non-human information

A total of two LLNAs and four GPMTs were identified testing skin sensitising effects of farnesol. EC3 values were 5.5 and 4.1%. In the GPMTs no positive reactions were observed after intradermal induction doses of 0.16, 5 and 10% farnesol.

No relevant *in vitro* studies on farnesol (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

5.6.1.3 Exposure

According to data from IFRA (2006) the exposure of farnesol when used as fragrance in cosmetics and in other consumer products appears to be low.

5.6.2 Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 0.35 and 0.9% i.e. all 4 studies reporting frequencies lower than 1%. For selected dermatitis patients positive reactions range between 0.02 and 13.2% with 7 out of 16 studies reporting frequencies higher than 2%. In addition to this there are more than 250 published cases of positive patch test reactions to farnesol. According to the CLP criteria a frequency $\geq 1\%$ for unselected/consecutive dermatitis patients and/or $\geq 2\%$ for selected dermatitis patients and/or a total number of published cases ≥ 100 , equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that farnesol causes a low/moderate frequency of occurrence of skin sensitisation based on unselected/consecutive dermatitis patients and 9/16 studies with selected dermatitis patients. The remaining studies with selected dermatitis patients (7/16) and number of published cases shows that farnesol causes a high frequency of occurrence of skin sensitisation in humans.

In regard to the HRIPT/HMT data the positive response reported at $> 500 \mu\text{g}/\text{cm}^2$ for HRIPT/HMT induction threshold indicate classification of farnesol in sub-category 1B according to Annex I:

3.4.2.2.2.2.

In the two LLNAs the lowest EC3 value for farnesol was 4.1%. According to the CLP Regulation an EC3 value $\leq 2\%$ indicates classification of a substance in sub-category 1A whereas an EC3 value $> 2\%$ indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.). Thus, both studies indicate classification of farnesol in sub-category 1B.

Sensitisation was not observed in the four GPMTs with intradermal induction doses of 0.16, 5 and 10% farnesol which do not justify sub-categorisation (Table 3.4.3).

Overall, there is evidence for classification in sub-category 1A based on the number of cases combined with the estimated low exposure. Data from HRIPT/HMT indicate evidence for sub-

category 1B. Data from LLNAs indicate a classification in sub-category 1B. A classification as a skin sensitiser in sub-category 1A is warranted for farnesol.

5.6.3 Conclusions on classification and labelling

Based on the number of cases combined with the estimated low exposure, a classification of farnesol as a skin sensitiser in sub-category 1A is justified.

5.7 Geraniol

5.7.1 Summary of the available data

5.7.1.1 Human data

A total of 84 results from patch test population studies, 7 HRIPTs, 4 HMTs and 2 case studies were identified with geraniol. The positive patch test frequencies from all of the reported patch test population studies vary between 0 and 40% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0 and 1.2% (10 studies) and in studies with selected dermatitis patients positive reactions range between 0 and 40% (74 studies). The total number of published cases is > 900. Sensitisation was reported in 2/4 HRIPT studies after exposure to 10% geraniol (11 810 µg/cm²) and in 1/4 HMT studies after exposure to 4140 µg/cm².

5.7.1.2 Non-human information

A total of 9 LLNAs, 5 GPMTs and 1 Buehler test were identified testing skin sensitising effects of geraniol. The reported EC3 values for geraniol ranged between 5.6% and 25.8% in different vehicles. In the GPMTs sensitisation was observed but not quantified (i.e. number of animals affected) in 4/5 studies with intradermal induction doses of 0.1, 5 and 10% geraniol. No sensitisation was observed in 1/5 GPMTs with an induction concentration of 50% geraniol and in the Buehler test with an induction concentration of 15% geraniol.

No relevant *in vitro* studies on geraniol (i.e. OECD TG 442C and OECD TG 442D) were identified in the literature.

5.7.1.3 Exposure

According to data from IFRA (2007) the exposure of geraniol when used as fragrance in cosmetics and in other consumer products appears to be relatively low. A recent study has indicated that up to 0.86% of the population might be exposed to geraniol from personal care products and household cleaning agents at levels exceeding the estimated Acceptable Exposure Level of 55 µg/cm² (Nijkamp et al., 2015).

5.7.2 Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 0 and 1.2% with 2/10 studies reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 0 and 40% with 44 out of 74 studies reporting frequencies higher than 2%. In addition to this there are more than 900 published cases of positive patch test reactions to geraniol. According to the CLP criteria a frequency ≥ 1% for unselected/consecutive dermatitis patients and/or ≥ 2% for selected dermatitis patients and/or a total number of published cases ≥ 100, equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that geraniol causes a *high frequency* of occurrence of skin sensitisation based on patch test data mainly from selected dermatitis patients and the number of published cases.

In regard to the HRIPT/HMT data the positive response reported at > 500 µg/cm² for HRIPT/HMT induction threshold indicate classification of geraniol in sub-category 1B according to Annex I: 3.4.2.2.2.2.

In the LLNAs EC3 values between 5.6 (vehicle: ethanol) and 25.8% (vehicle: ethanol:diethyl phthalate 1:3) were reported for geraniol. According to the CLP Regulation an EC3 value larger than 2% indicates classification of geraniol in sub-category 1B.

In the GPMTs sensitisation was reported to be observed but not quantified (i.e. number of animals affected) in 4/5 studies with intradermal induction doses of 0.1, 5 and 10% geraniol, therefore, these GPMTs cannot be compared with the classification criteria.

No sensitisation was observed in the Buehler test with an induction concentration of 15% geraniol. Overall, there is clear evidence for classification in sub-category 1A based on the frequency of sensitisation in human patch test studies mainly with selected dermatitis patients and the total number of cases combined with the estimated relatively low exposure. Data from HRIPT/HMT indicate evidence for sub-category 1B. LLNAs indicate a classification in sub-category 1B. A classification as a skin sensitiser in sub-category 1A is warranted for geraniol.

5.7.3 Conclusions on classification and labelling

Based on the high frequency of sensitisation observed in human patch test studies and the high number of published cases, combined with the estimated relatively low exposure, a classification of geraniol as a skin sensitiser in sub-category 1A is justified.

5.8 7-Hydroxycitronellal

5.8.1 Summary of the available data

5.8.1.1 Human data

A total of 39 results from patch test population studies, 4 modified HRIPTs, 15 HMTs and 3 case studies were identified with 7-hydroxycitronellal. The positive patch test frequencies from all of the reported patch test population studies vary between 0 and 55% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0.9 and 2.6% (4 studies) and in studies with selected dermatitis patients positive reactions range between 0 and 55% (35 studies). The total number of published cases is > 800. A LOEL-HRIPT/HMT (induction) of 5906 µg/cm² was established for 7-hydroxycitronellal by the RIFM Expert Panel.

5.8.1.2 Non-human information

A total of 7 LLNAs including 1 LLNA *ex vivo* BrdU, 1 GPMT and 1 Buehler test were identified testing skin sensitising effects of 7-hydroxycitronellal. The reported EC3 values for 7-hydroxycitronellal range between 9.8 and 33%. In the GPMTs sensitisation was observed but not quantified (i.e. number of animals affected) in 3/6 studies with intradermal induction doses of 0.4, 5 and 10% citral. In the GPMT sensitisation in 60% of the animals (number of animals not reported) after an intradermal induction dose of 0.5% 7-hydroxycitronellal. Sensitisation was also observed in 38% of the animals in the Buehler test with an induction concentration of 30% 7-hydroxycitronellal.

No relevant *in vitro* studies on 7-hydroxycitronellal (i.e. OECD TG 442C and OECD TG 442D) were identified in the literature.

5.8.1.3 Exposure

According to data from IFRA (2013c) the exposure of 7-hydroxycitronellal when used as fragrance in cosmetics and in other consumer products appears to be low.

5.8.2 Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 0.9 and 2.6% with 1/4 studies reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 0 and 55% with 29 out of 33 studies reporting frequencies equal to or higher than 2%. In addition to this there are more than 800 published cases of positive patch test reactions to 7-hydroxycitronellal. According to the CLP criteria a frequency ≥ 1% for unselected/consecutive dermatitis patients and/or ≥ 2% for selected dermatitis patients and/or a total number of published cases ≥ 100, equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b). The collected data described above from patch test studies show that 7-hydroxycitronellal causes a *high frequency* of occurrence of skin sensitisation based on these three types of information. In regard to the HRIPT/HMT data the positive response reported at > 500 µg/cm² for HRIPT/HMT induction threshold indicate evidence for sub-category 1B according to Annex I: 3.4.2.2.2.2.

In the seven LLNAs EC3 values between 9.8 and 33% were reported for 7-hydroxycitronellal. According to the CLP Regulation an EC3 value ≤ 2% indicates classification of a substance in sub-category 1A whereas an EC3 value > 2% indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.). Thus, all seven studies indicate classification of 7-hydroxycitronellal in sub-category 1B.

In the GPMT sensitisation was observed in 60% of the animals after an intradermal induction dose of 0.5% 7-hydroxycitronellal. According to the CLP criteria a positive response ≥ 60% of the animals responding at >0.1% to ≤ 1% intradermal induction dose indicates classification of a substance in

sub-category 1A (Annex I: 3.4.2.2.3.2.) and thus, this study indicates classification of 7-hydroxycitronellal into sub-category 1A.

Sensitisation was also observed in 38% of the animals in a Buehler test with an induction concentration of 30% 7-hydroxycitronellal. According to the CLP criteria a positive response $\geq 15\%$ of the animals responding at $>20\%$ topical induction dose indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.3.) and thus, this study indicates classification of 7-hydroxycitronellal into sub-category 1B.

Overall, there is clear evidence for classification in sub-category 1A based on the human patch test studies showing a high frequency of occurrence of skin sensitisation and the total number of cases combined with the estimated low exposure. Except from the GMTP study, which supports a sub-category 1A classification, the remaining animal studies (LLNA and Buehler) indicate a classification in sub-category 1B. A classification as a skin sensitiser in sub-category 1A is thus warranted for 7-hydroxycitronellal.

5.8.3 Conclusions on classification and labelling

Based on the high frequency of sensitisation observed in human patch test studies and the high number of published cases combined with the estimated low exposure a classification of 7-hydroxycitronellal as a skin sensitiser in sub-category 1A is justified.

5.9 Methyl oct-2-ynoate

5.9.1 Summary of the available data

5.9.1.1 Human data

A total of 11 results from patch test population studies and 5 case studies were identified with methyl oct-2-ynoate. The positive patch test frequencies from all of the reported patch test population studies vary between 0 and 2.9% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0 and 1.67% (3 studies) and in studies with selected dermatitis patients positive reactions range between 0.1 and 2.9% (8 studies). The total number of published cases is > 25. A LOEL-HRIPT/HMT (induction) of 194 µg/cm² was established for methyl oct-2-ynoate by the RIFM Expert Panel based on unpublished reports.

5.9.1.2 Non-human information

A total of 2 LLNAs (OECD TG 429), 1 GPMT and 1 Buehler test were identified testing skin sensitising effects of methyl oct-2-ynoate. In both LLNA studies an EC3 value of <0.5% was reported. In the GPMT sensitisation was observed in 90 % of the animals after an intradermal induction dose of 0.625% methyl oct-2-ynoate. Sensitisation was also observed in the Buehler test with positive reactions in 45-70% of the animals after an induction dose of 2.5%.

No relevant *in vitro* studies on methyl oct-2-ynoate (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

5.9.1.3 Exposure

According to data from IFRA (2008d) the exposure of methyl oct-2-ynoate when used as fragrance in cosmetics and in other consumer products appears to be low.

5.9.2 Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 0 and 1.67% with 1/3 studies reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 0.1 and 2.9% with 1 out of 8 studies reporting frequencies higher than 2%. In addition to this there are more than 25 published cases of positive patch test reactions to methyl oct-2-ynoate. According to the CLP criteria a frequency ≥ 1% for unselected/consecutive dermatitis patients and/or ≥ 2% for selected dermatitis patients and/or a total number of published cases ≥ 100, equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that methyl oct-2-ynoate causes a *low/moderate frequency* of occurrence of skin sensitisation based on these three types of information. In regard to HRIPT studies positive responses were observed at exposure to 194 µg/cm² methyl 2-ocynoate. A positive response at ≤ 500 µg/cm² in a HRIPT or HMT suggests categorisation into sub-category 1A according to Annex I: 3.4.2.2.2.1 and 3.4.2.2.2.2.

In the 2 LLNAs EC3 values were <0.5%. According to the CLP Regulation an EC3 value ≤ 2% indicates classification of a substance in sub-category 1A (Annex I: 3.4.2.2.3.2.).

In the GPMT sensitisation was observed in 90% of the animals after an intradermal induction dose of 0.625%. According to the CLP criteria a positive response ≥ 60% of the animals responding at > 0.1% to ≤ 1% intradermal induction dose indicates classification of a substance in sub-category 1A (Annex I: 3.4.2.2.3.2.).

In the Buehler test sensitisation was observed in 45-70% of the animals after an induction dose of 2.5%. According to the CLP criteria a positive response ≥ 60% of the animals responding at > 0.2%

to $\leq 20\%$ topical induction dose indicates classification of a substance in sub-category 1A (Annex I: 3.4.2.2.3.2.).

Overall, there is clear evidence for classification in sub-category 1A based on the very low EC3 values from the LLNAs. The results from GPMT and the Buehler test also supports sub-category 1A. Data from human patch test studies and the number of published cases justify classification of methyl oct-2-ynate in sub-category 1B while data from HRIPT studies justify classification of methyl oct-2-ynate in sub-category 1A. A classification as a skin sensitiser in sub-category 1A is thus warranted for methyl oct-2-ynate.

5.9.3 Conclusions on classification and labelling

Based on HRIPT data, the very low EC3 value from LLNAs and results from GPMT and Buehler tests a classification of methyl oct-2-ynate as a skin sensitiser in sub-category 1A is justified.

5.10 *Cinnamomum cassia* leaf oil / *Cinnamomum zeylanicum*, ext.

5.10.1 Summary of the available data

5.10.1.1 Human data

A total of 27 positive cases and frequencies between 1 and 27.8% in selected dermatitis patients tested with “cassia” essential oil or “cinnamon oil” were observed.

5.10.1.2 Non-human information

No animal studies with *Cinnamomum cassia* leaf oil / *Cinnamomum zeylanicum*, ext. have been identified.

5.10.1.3 Exposure

It has not been possible to identify any data on exposure to *Cinnamomum cassia* leaf oil or *Cinnamomum zeylanicum*, ext.

5.10.2 Comparison with criteria

One out of two studies with “cassia” essential oil gave a frequency of positive patch tests in selected patients of 27.8% i.e. $\geq 2\%$, which indicate categorisation into sub-category 1A.

No animal studies with *Cinnamomum cassia* leaf oil / *Cinnamomum zeylanicum*, ext. have been identified.

5.10.3 Conclusions on classification and labelling

Data on *Cinnamomum cassia* leaf oil / *Cinnamomum zeylanicum*, ext. alone is insufficient for sub-categorisation of *Cinnamomum cassia* leaf oil / *Cinnamomum zeylanicum*, ext. according to CLP criteria. It may be possible to sub-categorise *Cinnamomum cassia* leaf oil / *Cinnamomum zeylanicum*, ext. based on their constituents by read across to the major compounds such as cinnamaldehyde (for Cassia bark extract, Cassia oil, Cinnamon bark extract and Cinnamon bark oil) and eugenol (for Cinnamon leaf oil).

5.11 *Evernia prunastri* extract

5.11.1 Summary of the available data

5.11.1.1 Human data

A total of 35 results from patch test population studies, 5 HRIPTs and 2 case studies were identified with *Evernia prunastri* ext. The positive patch test frequencies from all of the reported patch test population studies vary between 0 and 64% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 1.8 and 6.8% (7 studies) and in studies with selected dermatitis patients positive reactions range between 0 and 64% (28 studies). The total number of published cases is > 1900. A LOEL-HRIPT/HMT (induction) of 1417 µg/cm² was established for *Evernia prunastri* ext. by the RIFM Expert Panel based on unpublished reports.

5.11.1.2 Non-human information

A total of 1 LLNA, 1 LLNA *ex vivo* BrdU and 1 GPMT were identified testing skin sensitising effects of *Evernia prunastri* ext. The reported EC3 value for *Evernia prunastri* ext. was 3.9% in the LLNA and 3.4% in the LLNA *ex vivo* BrdU. In the GPMT sensitisation was observed in 63% of the animals after an intradermal induction dose of 20% *Evernia prunastri* ext.

No relevant *in vitro* studies on *Evernia prunastri* ext. (i.e. OECD TG 442C and OECD TG 442D) were identified in the literature.

5.11.1.3 Exposure

According to data from IFRA (2008e) the exposure of *Evernia prunastri* ext. when used as fragrance in cosmetics and in other consumer products appears to be low.

5.11.2 Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 1.81 and 6.8% i.e. all studies are reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 0 and 64% with 23 out of 28 studies reporting frequencies higher than 2%. In addition to this there are more than 1900 published cases of positive patch test reactions to *Evernia prunastri* ext. According to the CLP criteria a frequency ≥ 1% for unselected/consecutive dermatitis patients and/or ≥ 2% for selected dermatitis patients and/or a total number of published cases ≥ 100, equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that *Evernia prunastri* ext. causes a *high frequency* of occurrence of skin sensitisation based on these three types of information.

In regard to the HRIPT/HMT data the positive response reported at > 500 µg/cm² for HRIPT/HMT induction threshold indicate evidence for sub-category 1B according to Annex I: 3.4.2.2.2.2.

In the 2 LLNAs EC3 values were 3.4 and 3.9%. According to the CLP Regulation an EC3 value ≤ 2% indicates classification of a substance in sub-category 1A whereas an EC3 value > 2% indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.). Thus, both studies indicate classification of *Evernia prunastri* ext. in sub-category 1B.

In the GPMT sensitisation was observed in 63% of the animals after an intradermal induction dose of 20%. According to the CLP criteria a positive response ≥ 30% of the animals responding at > 1% intradermal induction dose indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.) and thus, this study indicates classification of *Evernia prunastri* ext. into sub-category 1B.

Overall, there is clear evidence for classification in sub-category 1A based on the human patch test studies showing a high frequency of occurrence of skin sensitisation and the total number of cases

combined with the estimated low exposure. The results from the animal studies indicate classification in sub-category 1B. A classification as a skin sensitiser in sub-category 1A is thus warranted for *Evernia prunastri* ext.

5.11.3 Conclusions on classification and labelling

Based on the high frequency of sensitisation observed in human patch test studies and the high number of published cases combined with the estimated low exposure, a classification of *Evernia prunastri* ext. as a skin sensitiser in sub-category 1A is justified.

5.12 Conclusion

For all 11 substances selected for detailed assessment in Phase 2, a classification as a skin sensitiser in sub-category 1A is justified based on the available data.

For nine of the substances, sub-category 1A is justified based on human patch test data i.e. frequencies in unselected and/or selected dermatitis patients and/or a high number of cases. For methyl oct-2-ynate, sub-category 1A is justified primarily based on non-human data (very low EC3 values in the two LLNAs and results from GPMT and Buehler tests, and supported by human evidence from HRIPT studies).

For *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext.), sub-category 1A is justified based on their constituents by read across to the major compounds such as cinnamaldehyde (for Cassia bark extract, Cassia oil, Cinnamon bark extract and Cinnamon bark oil) and eugenol (for Cinnamon leaf oil).

6. Discussion

6.1 Sub-category 1A candidates (the 11 substances in Phase 2)

For all 11 substances selected for detailed assessment in Phase 2, a classification as a skin sensitiser in sub-category 1A is justified based on the available data.

Generally, sub-category 1A is justified based on the available human data:

For nine of the 11 substances selected for detailed assessment in Phase 2 (i.e. not including methyl oct-2-ynate and *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext.), sub-category 1A is justified based on human patch test data i.e. frequencies in unselected and/or selected dermatitis patients and/or a high number of cases. For two of the substances, farnesol and coumarin, sub-category 1A is justified solely by the high number of cases from human diagnostic patch test studies. For human diagnostic patch test data, only one or two types of information in terms of frequency in defined populations / number of cases may be sufficient for sub-categorisation. Combined with the estimated low exposure for all these substances it seems clear that sub-category 1A is justified for these nine substances based on the available data.

Generally, when sub-category 1A is justified based on the available human data this is not supported by non-human data:

For only two of the nine substances where sub-category 1A is justified based on the available human data, sub-category 1A is also supported by non-human data. For cinnamaldehyde, 21/22 LLNAs showed an EC3 value <2% and thus, justifying sub-category 1A. For 7-hydroxycitronellal, one GPMT supported sub-category 1A, whereas 7 LLNAs and one Buehler test indicated a classification in sub-category 1B.

For the remaining substances, non-human data, if available, do not support sub-category 1A. As a substance may be allocated to sub-category 1A on the basis of reliable and good quality evidence from human cases or epidemiological studies and/or observations from appropriate studies in experimental animals it seems clear that sub-category 1A is justified for these nine substances based on the available data, i.e. despite that the available non-human data do not support classification in sub-category 1A.

The fact that data from human and non-human studies only support each other for a few substances may be explained by the difference in exposure. As described by Anderson et al. (2011) the elicitation thresholds may be lower than those required for induction and the dose required for induction may depend on duration frequency and site of exposure. Humans could be exposed to these substances repeatedly, thus causing a relatively weak sensitiser, as measured by the EC3-value, to be of greater risk for allergic contact dermatitis in humans due to frequent exposure.

Only in some cases, sub-category 1A is justified based on the available non-human data:

Only for one of the 11 substances selected for detailed assessment in Phase 2, methyl oct-2-ynate, sub-category 1A is justified primarily based on non-human data, especially a very low EC3 value in the two LLNAs, but also the results from GPMT and Buehler tests. Furthermore, sub-category 1A is supported by human evidence in form of results in the HRIPT studies.

The reason for the low/moderate frequency of occurrence of skin sensitisation in human diagnostic patch test data with methyl oct-2-ynate is probably the observation that patch test reactions after methyl oct-2-ynate occurs relatively late (after 2-4 weeks) indicating active sensitisation (i.e. the subjects were sensitised by the patch test) (Heisterberg et al. 2010).

Sub-category 1A is justified based on read across:

For one of the 11 substances selected for detailed assessment in Phase 2, *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext. it is not possible to suggest a classification in sub-category 1A based on the limited available data. In contrast to the other 10 substances selected for detailed assessment in Phase 2, this fragrance is a mixture of substances and the available data did not include specific information on composition and purity of the constituents in the fragrance. It may, however, be possible to sub-categorise *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext. based on their constituents by read across to the major constituents, i.e. cinnamaldehyde (for Cassia bark extract (44%), Cassia oil (87%), Cinnamon bark extract (38%) and Cinnamon bark oil (75%)) and eugenol (for Cinnamon leaf oil (70%)). Both cinnamaldehyde and eugenol are among the 11 substances selected for detailed assessment in Phase 2 for which sub-category 1A is justified. Based on a read across it seems clear that sub-category 1A is justified for *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext. based on the available data.

6.2 Other possible sub-category 1A candidates (9 substances given priority 1 in Phase 1)

In addition to the 11 substances selected for detailed assessment in Phase 2, nine other substances (butyl methacrylate, limonene, turpentine oil, Ylang ylang ext./oil, clove leaf oil, *Evernia furfuracea* ext., jasmine, Peru balsam, Sandalwood ext./oil) were given a priority 1 in Phase 1, i.e. considered as possible sub-category 1A candidates.

Seven of these substances (i.e. not including butyl methacrylate and limonene) were considered as possible sub-category 1A candidates based on human patch test data, i.e. frequencies in unselected and/or selected dermatitis patients and/or a high number of cases.

Limonene was considered as a possible sub-category 1A candidate as it is among the substances considered by SCCS (2012) as a substance of special concern, defined as due to the high number of reported cases (>100 cases).

Butyl methacrylate was considered as a possible sub-category 1A candidate based on high frequency of occupational allergic contact dermatitis due to (meth)acrylates in general.

Overall, all these nine substances were considered as possible sub-category 1A candidates based on the available human data. A detailed assessment of these substances was, however, not part of the current project.

6.3 Remaining substances (22 substances given priority 2 in Phase 1)

The remaining 22 substances selected for the preliminary assessment in Phase 1 were given a priority 2, i.e. not considered as possible sub-category 1A candidates.

For 5 substances (d-limonene, (l-limonene), carvone, HICC, isoeugenol), a proposal for a harmonised classification as skin sensitiser in Category 1 H317, or sub-category 1A or 1B has already been submitted.

For 10 substances (benzaldehyde, 2-methyl-4-phenylpentanol, acetylcedrene, trans-anethole, anise alcohol, benzyl salicylate, 2-(4-tert-butylbenzyl)propionaldehyde, citronellol, peppermint ext./oil, spearmint ext.), sub-category 1A is not justified based on human patch test data, i.e. due to a low frequency and low number of cases in the human studies, as well as no/equivocal sensitisation was noted in the non-human studies.

For the remaining 7 substances (mixture (of trans-4-acetoxy-4-methyl-2-propyl-tetrahydro-2H-pyran; cis-4-acetoxy-4-methyl-2-propyl-tetrahydro-2H-pyran), Damascone, pin-2(3)-ene/pin-2(10)-ene, p-mentha-1,4(8)-diene, Neroli oil / Orange sour ext., lemon ext., orange sweet ext.), the

available data were too limited for an indication as a possible sub-category 1A candidate. Thus, it cannot be excluded whether these substances would turn out to be sub-category 1A candidates should additional human studies and/or non-human data become available.

6.4 Exposure assessment

One of the elements in the criteria for classification in sub-category 1A based on human patch test data is 'relatively low exposure'. Relatively high or relatively low exposure relates to the concentrations of the substances in cosmetics and other consumer products which individuals are exposed to in their daily lives, in the workplace, or other conditions and resulting in sensitisation. A cut-off concentration of 1 % has been set in order to discriminate between relatively high exposure (≥ 1.0 %) and relatively low exposure (< 1.0 %).

For the 11 substances selected for Phase 2, relatively high or relatively low exposure from use as fragrance in cosmetics and in other consumer products has been evaluated based on the IFRA standard limit of each substance in each of the 11 IFRA QRA product categories (IFRA, 2015), i.e., if the IFRA standard limit is < 1.0 % the exposure is thus considered as being relatively low. This is generally the case for the 11 Phase 2 substances for most of the IFRA QRA product categories where the IFRA standard limits have been set based on QRA.

The SCCS has recently published a 'Memorandum on use of Human Data in risk assessment of skin sensitisation' (SCCS, 2015). It is mentioned that the data used in the QRA approach is animal data and/or results from predictive tests in humans, i.e., experimental induction tests such as the Human Repeated Insult Patch Test (HRIPT) and the Human Maximization Test (HMT). However, it is also mentioned that it would be more appropriate to consider the epidemiological and diagnostic patch test data as these represent the relevant end-point. Furthermore, it is noted that the QRA approach is still under evaluation.

If the QRA approach would be revised in order to include also the epidemiological and diagnostic patch test data the current IFRA standard limits based on the QRA approach might change. If the current IFRA standard limits below 1 % would change to values above this cut-off concentration for relatively high/low exposure this could have an impact on the classification as a skin sensitizer in sub-category 1A for the 11 Phase 2 substances.

6.5 Data quality

A relatively large proportion of the cited studies have only been available from secondary sources, mainly attributed to unpublished data by the industry. Inclusion of these studies in the preliminary assessment of the 42 selected substances in Phase 1, as well as for the detailed assessment of the 11 substances in Phase 2 is justified due to the purpose of the current project, i.e. a screening of the available data for a preliminary assessment whether the criteria for classification in sub-category 1A according to the CLP criteria are fulfilled.

Secondary sources have a reliability score 4 "not assignable" according to the reliability criteria proposed by Klimisch et al. (1997). For a genuine assessment of studies only available from secondary sources, the original study report should be available for the assessment.

6.6 Conclusion

For all 11 substances selected for detailed assessment in Phase 2, a classification as a skin sensitiser in sub-category 1A is justified based on the available data.

Based on the assessments in this project, a decision whether a classification of a substance as a skin sensitiser in sub-category 1A is justified is generally based on the available human data.

For the 11 substances selected for Phase 2, relatively low exposure from use as fragrance in cosmetics and in other consumer products has been evaluated based on the IFRA standard limit of each substance in each of the 11 IFRA QRA product categories, i.e., if the IFRA standard limit is < 1.0 % the exposure is thus considered as being relatively low. This is generally the case for the 11 substances for most of the IFRA QRA product categories where the IFRA standard limits have been set based on QRA. Data for the QRA are generally animal data and/or results from predictive tests in humans (HRIPT and HMT). As it would be more appropriate to consider the epidemiological and diagnostic patch test data the current IFRA standard limits might change if the QRA approach would be revised in order to include also the epidemiological and diagnostic patch test data. This could have an impact on the classification as a skin sensitiser in sub-category 1A for the 11 substances if the current IFRA standard limits below 1 % would change to values above this cut-off concentration for relatively high/low exposure.

A relatively large proportion of the cited studies have only been available from secondary sources, mainly attributed to unpublished data by the industry. For a genuine assessment of such studies, the original study reports should be available for the assessment. Thus, it cannot be excluded whether the outcome of the assessments performed in this project could turn out differently should the original study reports become available for the final assessment.

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Appendix 1 List of abbreviations and acronyms

AEL	Acceptable Exposure Level
AOO	Acetone:Olive oil
BHT	Butylated hydroxytoluene
BrdU-ELISA	5-Bromo-2-deoxyuridine-enzyme-linked immunosorbent assay
CAS RN	Chemical Abstract Service Registration Number
DEP	Diethyl Phthalate
DMF	Dimethylformamide
DMSO	Dimethyl Sulfoxide
FM	Fragrance mix
EC3 value	Effective Concentration inducing a stimulation index of 3 in the LLNA test.
EtOH	Ethanol
FM	Fragrance Mix
GPMT	Guinea Pig Maximisation Test
IFRA	International Fragrance Association
LLNA	Local Lymph Node Assay
MEK	Methyl ethyl ketone
NESIL	No Expected Sensitization Induction Level
OECD TG	Organisation for Economic Co-operation and Development Test Guideline
PEG	Polyethylene glycol
Pet.	Petrolatum
QRA	Quantitative Risk Assessment
REACH RD	Registration, Evaluation, Authorisation and Restriction of Chemicals Registration Dossier
RIFM	Research Institute for Fragrance Materials, Inc.
SAF	Sensitisation Assessment Factors
SCCNFP	Scientific Committee on Cosmetic products and Non-Food Products intended for consumers
SCCS	Scientific Committee on Consumer Safety
SSO	Sorbitan sesquioleate
WoE	Weight of Evidence

Appendix 2 List of substances for evaluation in Phase 1

CAS	Stofnavn	Skin sens 1 Harm. Klass.	Skin sens 1 Selvkl. (også 1A eller 1B)	Andel virks. med Skin sens. selvkl. (ca.)	REACH registr.	Prioritet
97-88-1	Butyl methacrylate	x	x		Ja	1a
100-52-7	Benzaldehyde		x	>90%	Ja	1a
5392-40-5	Citral	x	x		Ja	1a
5989-27-5	(R)-p-mentha-1,8-diene (d-limonene)	x	x		Ja	1a
5989-54-8	(S)-p-mentha-1,8-diene (l-limonene)	x	x		Ja	1a
92585-24-5	2-methyl-4-phenylpentanol	x	x		Ja	1a
131766-73-9	A mixture of: trans-4-acetoxy-4-methyl-2-propyl-tetrahydro-2H-pyran; cis-4-acetoxy-4-methyl-2-propyl-tetrahydro-2H-pyran	x	x		Ja	1a
8006-64-2	Turpentine oil	x	x		Ja	1a
32388-55-9	[3R-(3 α ,3 β ,7 β ,8 α)]-1-(2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-1H-3a,7-methanoazulen-5-yl)ethan-1-one (Acetylcedrene)		x	>90%	Ja	1a
4180-23-8	(E)-anethole (trans-anethole)		x	>90%	Ja	1a
105-13-5	4-methoxybenzyl alcohol (Anise alcohol)		x	>90%	Ja	1a
118-58-1	Benzyl salicylate		x	>90%	Ja	1a
80-54-6	2-(4-tert-butylbenzyl)propionaldehyde (Butylphenyl methylpropional)		x	>90%	Ja	1a
99-49-0/	d-p-mentha-1(6),8-dien-2-one (Carvone) /		x	>90%	Nej	2

CAS	Stofnavn	Skin sens 1 Harm. Klass.	Skin sens 1 Selvkl. (også 1A eller 1B)	Andel virks. med Skin sens. selvkl. (ca.)	REACH registr.	Prioritet
6485-40-1/	l-p-mentha-1(6),8-dien-2-one (Carvone) /		x	>90%	Ja	1a
2244-16-8	(S)-2-methyl-5-(1-methylvinyl)cyclohex-2-en-1-one (Carvone)		x	ca 50%	Nej	2
104-55-2	cinnamaldehyde		x	>90%	Ja	1a
104-54-1	cinnamyl alcohol		x	>90%	Ja	1a
106-22-9/	citronellol		x	>90%	Ja	1a
1117-61-9/	(R)-3,7-dimethyloct-6-en-1-ol		x	>90%	Nej	2
7540-51-4	(-)-3,7-dimethyloct-6-en-1-ol		x	>85%	Nej	2
91-64-5	coumarin		x	>90%	Ja	1a
57378-68-4	1-(2,6,6-trimethyl-3-cyclohexen-1-yl)-2-buten-1-one (delta-DAMASCONE)		x	100%	Nej	1b
97-53-0	eugenol		x	>90%	Ja	1a
4602-84-0	farnesol		x	>85%	Ja	1a
106-24-1	geraniol		x	>90%	Ja	1a
31906-04-4/	4-(4-hydroxy-4-methylpentyl)cyclohex-3-enecarbaldehyde (Hydroxyisoheptyl 3-cyclohexene carboxaldehyde, HICC)		x	>90%	Nej	1d
51414-25-6			x	100%	Nej	1d
107-75-5	7-hydroxycitronellal		x	100%	Ja	1a
97-54-1	isoeugenol		x	100%	Nej	1b
138-86-3	limonene	x	x		Nej	1c
111-12-6	methyl oct-2-ynoate		x	>90%	Nej	1b
80-56-8/	pin-2(3)-ene /		x	>75%	Ja	1a
127-91-3	pin-2(10)-ene		x	>90%	Ja	1a

CAS	Stofnavn	Skin sens 1 Harm. Klass.	Skin sens 1 Selvklass. (også 1A eller 1B)	Andel virks. med Skin sens. selvkl. (ca.)	REACH registr.	Prioritet
586-62-9	p-mentha-1,4(8)-diene (terpinolene)		x	> 75%	Ja	1a
83863-30-3/	Ylang ylang ext. /		x	>90%	Nej	1d
8006-81-3	Ylang ylang oil		x	>90%	Nej	1d
8007-80-5/	Cinnamomum cassia leaf oil /		x	ca. 80%	Nej	2
84649-98-9	Cinnamomum zeylanicum, ext.		x	>90%	Ja	1a
8016-38-4/	Neroli Oil /		x	>95%	Nej	2
72968-50-4	Orange, sour, ext.		x	>95%	Ja	1a
84929-31-7	Lemon, ext.		x	>95%	Ja	1a
97766-30-8/	Orange, sweet, Valencia, ext. /		x	>95%	Nej	2
8028-48-6	Orange, sweet, ext.		x	>95%	Ja	1a
8000-34-8	Clove leaf oil		x	>80%	Nej	1d
90028-67-4	Evernia furfuracea, ext.		x	>95%	Nej	1d
90028-68-5	Evernia prunastri, ext.		x	>95%	Nej	1d
84776-64-7/	Jasmine, Jasminum grandiflorum, ext. /		x	>95%	Nej	1d
90045-94-6/	Jasmine, Jasminum officinale, ext. /		x	>95%	Nej	1d
8022-96-6	Extract Jasmine (oil), Jasmine, Jasminum grandiflorum, ext.		x	ca. 30%	Nej	1d
8006-90-4/	Oils, peppermint (Mentha piperita) /		x	ca. 65%	Nej	2
84082-70-2	Peppermint, ext.		x	ca. 25%	Ja	1??
84696-51-5	Spearmint, ext. (Mentha spicata)		x	>95%	Nej	2
8007-00-9	Balsams, Peru (Myroxylon pereirae)		x	>95%	Nej	1d
84787-70-2/	Sandalwood, ext. /		x	10 af >900	Nej	1d
8006-87-9	Sandalwood oil		x	1	Nej	1d

Appendix 3 Justification for the prioritisation of the substances

The justification for prioritisation of the substances into group 1 or 2 is based on studies identified in the literature search, REACH RD and in the SCCS (2012) opinion.

CAS RN	Name	Harm class	LLNA ^{a)}	GPMT/Buehler ^{b)}	Human diagnostic patch test data ^{c)}	Prioritisation
97-88-1	Butyl methacrylate	x	OECD TG 429: EC3 > 2% (1 study).	GPMT OECD TG 406: 80% responding after intradermal induction dose of 5% (1 study). Sensitization in 1/8 non TG.	Unselected dermatitis patients frequency ≥ 1% (0/1 study). Selected dermatitis patients frequency ≥ 2% (3/8 studies). Workplace selected workers frequency ≥ 1% (1/1 study). N _(cases) < 100.	1 Based on the high frequency of occupational allergic contact dermatitis among dentists, nail technicians, fibreglass workers etc. due to (meth)acrylates.
100-52-7	Benzaldehyde		OECD TG 429: Not sensitising (1 study).	Not sensitising in 3/3 GPMTs similar to TG.	Selected dermatitis patients frequency ≥ 2% (1/4 studies). N _(cases) < 100. Human maximisation test (HMT): No conclusion (1 study).	2 Based on the low frequency and number of cases in human studies and no sensitisation in non-human studies.
5392-40-5	Citral	x	OECD TG 429: EC3 ≤ 2% (2 studies). EC3 > 2% (8 studies).	GPMT similar to OECD TG 406: 60 and 100% responding after intradermal induction dose of 2% (2 studies).	Unselected dermatitis patients frequency ≥ 1% (5/11 studies). Selected dermatitis patients frequency ≥ 2% (6/8	1 Based on the high frequency and number of cases in human studies and sensitisation in non-human

CAS RN	Name	Harm class	LLNA ^{a)}	GPMT/Buehler ^{b)}	Human diagnostic patch test data ^{c)}	Prioritisation
					studies). N _(cases) > 100. Human Repeat Insult Patch Test (HRIPT): Mixed results (2 studies).	studies. SCCS substance of special concern ^{d)} .
5989-27-5	(R)-p-Mentha-1,8-diene (d-limonene)	x	OECD TG 429: EC3 > 2% (6 studies).	—	Unselected dermatitis patients frequency ≥ 1% (5/5 studies – two with mixed results). Selected dermatitis patients frequency ≥ 2% (6/8 studies). Workplace selected workers frequency ≥ 1% (1/1 studies). Workplace all or randomly selected workers frequency ≥ 0.4% (2/2 studies). N _(cases) < 100.	2 Harmonised classification sub-category 1B proposal.
5989-54-8	(S)-p-Mentha-1,8-diene (l-limonene)	x	—	—	Unselected dermatitis patients frequency ≥ 1% (1/1). N _(cases) < 100.	2 Read-across to d-limonene.
92585-24-5	2-methyl-4-phenylpentanol	x	OECD TG 429: Not sensitising (1 study).	Buehler test non TG: ambiguous.	Selected dermatitis patients frequency ≥ 2% (0/2 studies).	2 Based on the low frequency and number of cases in

CAS RN	Name	Harm class	LLNA ^{a)}	GPMT/Buehler ^{b)}	Human diagnostic patch test data ^{c)}	Prioritisation
					$N_{(\text{cases})} < 100$.	human studies and no sensitisation in non-human studies.
131766-73-9	A mixture of: trans-4-acetoxy-4-methyl-2-propyl-tetrahydro-2H-pyran; cis-4-acetoxy-4-methyl-2-propyl-tetrahydro-2H-pyran	x	OECD TG 429: Not sensitising (1 study).	GPMT non TG: 70% responding after intradermal induction dose of ?% (1 study).	—	2 Based on limited data.
8006-64-2	Turpentine oil	x	—	GPMT non TG: Sensitising.	General population studies frequency $\geq 0.2\%$ (1/1 study). Unselected dermatitis patients frequency $\geq 1\%$ (8/10 studies). Selected dermatitis patients frequency $\geq 2\%$ (1/1 study (prisoners)). Workplace selected workers frequency $\geq 1\%$ (1/1 studies). $N_{(\text{cases})} > 100$.	1 Based on the high frequency and number of cases in human studies. SCCS substance of special concern ^{d)} .
32388-55-9	[3R-(3 α ,3 $\alpha\beta$,7 β ,8 $\alpha\alpha$)]-1-(2,3,4,7,8,8a-Hexahydro-3,6,8,8-		OECD TG 429: EC3 > 2% (1 study).	—	Unselected dermatitis patients frequency $\geq 1\%$ (0/3 studies). $N_{(\text{cases})} < 100$.	2 Based on the low frequency and number of cases in human studies and no

CAS RN	Name	Harm class	LLNA ^{a)}	GPMT/Buehler ^{b)}	Human diagnostic patch test data ^{c)}	Prioritisation
	tetramethyl-1H-3a,7-methanoazulen-5-yl)ethan-1-one (Acetylcedrene)					sensitisation in non-human studies.
4180-23-8	(E)-Anethole (trans-anethole)		—	GPMT OECD TG 406(?): 100% responding after intradermal induction dose of 2% (1 study).	Two case stories. N _(cases) < 100.	2 Based on the low frequency and number of cases in human studies and one non-human study not warranting sub-category 1A.
105-13-5	4-Methoxybenzyl alcohol (Anise alcohol)		OECD TG 429: EC3 > 2% (1 study).	—	Unselected dermatitis patients frequency ≥ 1% (0/5 studies). N _(cases) < 100. Human maximisation test (HMT): Not sensitising (1 study).	2 Based on the low frequency and number of cases in human studies and no sensitisation in non-human studies.
118-58-1	Benzyl salicylate		OECD TG 429: EC3 > 2% (1 study).	—	Unselected dermatitis patients frequency ≥ 1% (1/9 studies). Selected dermatitis patients frequency ≥ 2% (2/2 studies). N _(cases) < 100.	2 Based on the low frequency and number of cases in human studies and no sensitisation in non-human studies.
80-54-6	2-(4-tert-		OECD TG 429:	GPMT OECD TG 406: Not	Unselected dermatitis	2

CAS RN	Name	Harm class	LLNA ^{a)}	GPMT/Buehler ^{b)}	Human diagnostic patch test data ^{c)}	Prioritisation
	Butylbenzyl)propionaldehyde (Butylphenyl methylpropional)		EC3 > 2% (6 studies).	sensitising (2 studies). GPMT non TG: Strong sensitiser (1 study).	patients frequency ≥ 1% (0/12 studies). Selected dermatitis patients frequency ≥ 2% (1/1 studies). N _(cases) < 100. Human maximisation test (HMT): Mixed results (1 study).	Based on the low frequency and number of cases in human studies and equivocal results from non-human studies.
99-49-0 / 6485-40-1 / 2244-26-8	d-p-Mentha-1(6),8-dien-2-one (Carvone) / l-p-Mentha-1(6),8-dien-2-one (Carvone) / (S)-2-Methyl-5-(1-methylvinyl)cyclohex-2-en-1-one (Carvone)		OECD TG 429: EC3 > 2% (3 studies).	—	Selected dermatitis patients frequency ≥ 2% (1/1 studies). N _(cases) < 100.	2 Harmonised classification category 1 proposal.
104-55-2	Cinnamaldehyde		OECD TG 429: EC3 ≤ 2% (19 studies) EC3 > 2% (1 study).	GPMT non TG: Sensitising (4 studies).	Unselected dermatitis patients frequency ≥ 1% (12/12 studies). Selected dermatitis patients frequency ≥ 2% (6/7 studies). Workplace all or randomly selected workers frequency ≥	1 Based on the high frequency and number of cases in human studies and sensitisation in non-human studies. SCCS substance of special

CAS RN	Name	Harm class	LLNA ^{a)}	GPMT/Buehler ^{b)}	Human diagnostic patch test data ^{c)}	Prioritisation
					0.4% (1/1 studies). N _(cases) > 100.	concern ^{d)} .
104-54-1	Cinnamyl alcohol		OECD TG 429: EC3 > 2% (5 studies).	GPMT OECD TG 406: 30% responding after intradermal induction dose of ?% (1 study). GPMT non TG: Not sensitising (1 study). Buehler OECD TG 406: Not sensitising after topical induction dose of ?% (1 study).	Unselected dermatitis patients frequency ≥ 1% (6/9 studies). Selected dermatitis patients frequency ≥ 2% (6/7 studies). N _(cases) > 100. Human maximisation test (HMT): Not sensitising (1 study). Human Repeat Insult Patch Test (HRIPT): Sensitising (1 study).	1 Based on the high frequency and number of cases in human studies. SCCS substance of special concern ^{d)} .
106-22-9 / 1117-61-9 / 7540-51-4	Citronellol / (R)-3,7-dimethyloct-6-en-1-ol / (-)-3,7-dimethyloct-6-en-1-ol		OECD TG 429: EC3 > 2% (1 study).	GPMT non TG: Not sensitising after topical induction dose of 6% (1 study). Buehler OECD TG 406: Not sensitising (1 study).	Unselected dermatitis patients frequency ≥ 1% (2/8 studies). Selected dermatitis patients frequency ≥ 2% (3/4 studies). N _(cases) < 100. Human maximisation test (HMT): Not sensitising (1 study). Human Repeat Insult Patch	2 Based on the low frequency and number of cases in human studies and no sensitisation in non-human studies.

CAS RN	Name	Harm class	LLNA ^{a)}	GPMT/Buehler ^{b)}	Human diagnostic patch test data ^{c)}	Prioritisation
					Test (HRIPT): Not sensitising (1 study).	
91-64-5	Coumarin		OECD TG 429: EC3 > 2% (1 study).	GPMT non TG: Not sensitising (1 study).	Unselected dermatitis patients frequency ≥ 1% (1/8 studies). Selected dermatitis patients frequency ≥ 2% (3/7 studies). N _(cases) < 100.	1 SCCS substance of special concern ^{d)} .
57378-68-4	1-(2,6,6-Trimethyl-3-cyclohexen-1-yl)-2-buten-1-one (delta-DAMASCONE)		OECD TG 429(?): EC3 > 2% (2 studies).	—	Human Repeat Insult Patch Test (HRIPT): Several positive and one negative study according to SCCS (2012). N _(cases) < 100.	2 Based on limited data.
97-53-0	Eugenol		OECD TG 429: EC3 > 2% (10 studies).	GPMT similar to OECD TG 406: 20% responding after intradermal induction dose of 5% (1 study).	Unselected dermatitis patients frequency ≥ 1% (7/11 studies). Selected dermatitis patients frequency ≥ 2% (6/6 studies). N _(cases) > 100.	1 Based on the high frequency and number of cases in human studies. SCCS substance of special concern ^{d)} .
4602-84-0	Farnesol		OECD TG 429: EC3 > 2% (2 studies).	—	Unselected dermatitis patients frequency ≥ 1% (1/7 studies – with a frequency of	1 Based on very high frequencies in a limited

CAS RN	Name	Harm class	LLNA ^{a)}	GPMT/Buehler ^{b)}	Human diagnostic patch test data ^{c)}	Prioritisation
					13%). Selected dermatitis patients frequency $\geq 2\%$ (2/4 studies – with frequencies of 10 and 12%). $N_{(cases)} > 100$.	number of studies and the number of cases in human studies. SCCS substance of special concern ^{d)} .
106-24-1	Geraniol		OECD TG 429: EC3 > 2% (5 studies).	GPMT non TG: Not sensitising in four studies. Sensitising in three studies.	Unselected dermatitis patients frequency $\geq 1\%$ (6/16 studies). Selected dermatitis patients frequency $\geq 2\%$ (3/7 studies). $N_{(cases)} > 100$. Human maximisation test (HMT): Not sensitising (2 studies).	1 Based on the high frequency and number of cases in human studies. SCCS substance of special concern ^{d)} .
31906-04-4 / 51414-25-6	4-(4-Hydroxy-4-methylpentyl)cyclohex-3-enecarbaldehyde (Hydroxyisohexyl 3-cyclohexene carboxaldehyde, HICC)	RAC 1A	OECD TG 429: EC3 > 2% (1 study).	—	Unselected dermatitis patients frequency $\geq 1\%$ (17/19 studies). Selected dermatitis patients frequency $\geq 2\%$ (3/3 studies). Workplace selected workers frequency $\geq 1\%$ (0/1 study). $N_{(cases)} > 100$.	Harmonised classification sub-category 1A proposal.
107-75-5	7-hydroxycitronellal		OECD TG 429: EC3 > 2% (7 studies).	GPMT non TG: 60% responding after	Unselected dermatitis patients frequency $\geq 1\%$ (7/8	1 Based on the high frequency

CAS RN	Name	Harm class	LLNA ^{a)}	GPMT/Buehler ^{b)}	Human diagnostic patch test data ^{c)}	Prioritisation
				intradermal induction dose of 0.5% (1 study). Buehler non TG: ambiguous.	studies). Selected dermatitis patients frequency $\geq 2\%$ (4/6 studies). $N_{(\text{cases})} > 100$.	and number of cases in human studies. SCCS substance of special concern ^{d)} .
97-54-1	Isoeugenol	RAC 1A	OECD TG 429: EC ₃ $\leq 2\%$ (17 studies) EC ₃ $> 2\%$ (6 studies).	—	Unselected dermatitis patients frequency $\geq 1\%$ (9/9 studies). Selected dermatitis patients frequency $\geq 2\%$ (8/8 studies). Workplace selected workers frequency $\geq 1\%$ (0/1 study). $N_{(\text{cases})} > 100$.	Harmonised classification sub-category 1A proposal.
138-86-3	Limonene	x	—	—	Unselected dermatitis patients frequency $\geq 1\%$ (3/10 studies). $N_{(\text{cases})} > 100$ (mainly due to hydroperoxides of limonene).	1 SCCS substance of special concern ^{d)} .
111-12-6	Methyl oct-2-ynoate		OECD TG 429: EC ₃ $\leq 2\%$ (2 studies).	—	Unselected dermatitis patients frequency $\geq 1\%$ (2/6 studies). Selected dermatitis patients frequency $\geq 2\%$ (0/1 studies). $N_{(\text{cases})} < 100$.	1 Based on the very low EC ₃ value in LLNAs.

CAS RN	Name	Harm class	LLNA ^{a)}	GPMT/Buehler ^{b)}	Human diagnostic patch test data ^{c)}	Prioritisation
80-56-8 / 127-91-3	Pin-2(3)-ene / Pin-2(10)-ene		OECD TG 429: EC3 > 2% (1 study). Test substance beta-pinene (CAS 127-91-2).	—	Selected dermatitis patients frequency ≥ 2% (2/2 studies). Workplace selected workers frequency ≥ 1% (1/1 studies). N _(cases) < 100.	2 Based on limited data.
586-62-9	p-Mentha-1,4(8)-diene (Terpinolene)		OECD TG 429: EC3 > 2% (1 study).	—	Selected dermatitis patients frequency ≥ 2% (1/1 study). N _(cases) < 100.	2 Based on limited data.
83863-30-3 / 8006-81-3	Ylang ylang ext. / Ylang ylang oil		OECD TG 429: EC3 > 2% (1 study).	—	Unselected dermatitis patients frequency ≥ 1% (9/10 studies). Selected dermatitis patients frequency ≥ 2% (6/7 studies). N _(cases) > 100.	1 Based on the high frequency and number of cases in human studies. SCCS substance of special concern ^{d)} .
8007-80-5 / 84649-98-9	<i>Cinnamomum cassia</i> leaf oil / <i>Cinnamomum zeylanicum</i> , ext.	—		—	Unselected dermatitis patients frequency ≥ 1% (1/1 study). Selected dermatitis patients frequency ≥ 2% (1/1 study). N _(cases) < 100.	1 Based on the high content of cinnamaldehyde, cinnamyl alcohol and/or eugenol (all SCCS substances of special concern ^{d)}).
8016-38-4 / 72968-50-4	Neroli Oil / Orange, sour, ext.	—		—	Unselected dermatitis patients frequency ≥ 1% (1/2 studies). Selected dermatitis patients	2 Based on limited data.

CAS RN	Name	Harm class	LLNA ^{a)}	GPMT/Buehler ^{b)}	Human diagnostic patch test data ^{c)}	Prioritisation
					frequency $\geq 2\%$ (2/2 studies). $N_{(\text{cases})} < 100$. Human maximisation test (HMT): Not sensitising (1 study).	
84929-31-7	Lemon, ext.	—	—	—	Unselected dermatitis patients frequency $\geq 1\%$ (0/1 studies). Selected dermatitis patients frequency $\geq 2\%$ (3/5 studies). $N_{(\text{cases})} < 100$.	2 Based on limited data.
97766-30-8 / 8028-48-6	Orange, sweet, Valencia, ext. / Orange, sweet, ext.	—	—	—	Unselected dermatitis patients frequency $\geq 1\%$ (0/3 studies). Selected dermatitis patients frequency $\geq 2\%$ (3/4 studies). $N_{(\text{cases})} < 100$.	2 Based on limited data.
8000-34-8	Clove leaf oil	—	—	—	Unselected dermatitis patients frequency $\geq 1\%$ (2/2 studies). Selected dermatitis patients frequency $\geq 2\%$ (3/5 studies). $N_{(\text{cases})} > 100$.	1 Based on the high frequency and number of cases in human studies. SCCS substance of special concern ^{d)} .
90028-67-4	<i>Evernia furfuracea</i> ,	OECD TG 429:	—	—	Unselected dermatitis	1

CAS RN	Name	Harm class	LLNA ^{a)}	GPMT/Buehler ^{b)}	Human diagnostic patch test data ^{c)}	Prioritisation
	ext.		EC3 > 2% (2 studies).		patients frequency ≥ 1% (5/5 studies). Selected dermatitis patients frequency ≥ 2% (2/2 studies). N _(cases) > 100.	Based on the high frequency and number of cases in human studies. SCCS substance of special concern ^{d)} .
90028-68-5	<i>Evernia prunastri</i> , ext.		OECD TG 429: EC3 > 2% (1 study).	—	Unselected dermatitis patients frequency ≥ 1% (10/10 studies). Selected dermatitis patients frequency ≥ 2% (6/6 studies). Workplace selected workers frequency ≥ 1% (0/1 studies). N _(cases) > 100.	1 Based on the high frequency and number of cases in human studies. SCCS substance of special concern ^{d)} .
84776-64-7 / 90045-94-6 / 8022-96-6	Jasmine, <i>Jasminum grandiflorum</i> , ext. / Jasmine, <i>Jasminum officinale</i> , ext. / Extract Jasmine (oil), Jasmine, <i>Jasminum grandiflorum</i> , ext.		OECD TG 429: EC3 > 2% (1 study).	—	Unselected dermatitis patients frequency ≥ 1% (5/7 studies). Selected dermatitis patients frequency ≥ 2% (7/11 studies). N _(cases) > 100.	1 Based on the high frequency and number of cases in human studies. SCCS substance of special concern ^{d)} .
8006-90-4 / 84082-70-2	Oils, peppermint (<i>Mentha piperita</i>) / Peppermint, ext.	—		GPMT non TG: 10-40% responding after intradermal induction dose of 0.25 -0.5% (4 studies).	Unselected dermatitis patients frequency ≥ 1% (0/3 studies). Selected dermatitis patients frequency ≥ 2% (3/5 studies).	2 Based on the low frequency and number of cases in human studies, and mixed results in the non-TG-non-

CAS RN	Name	Harm class	LLNA ^{a)}	GPMT/Buehler ^{b)}	Human diagnostic patch test data ^{c)}	Prioritisation
					N _(cases) < 100.	human studies.
84696-51-5	Spearmint, ext. (<i>Mentha spicata</i>)		–	–	Unselected dermatitis patients frequency ≥ 1% (0/1 study). Selected dermatitis patients frequency ≥ 2% (1/1 study). N _(cases) < 100.	2 Based on the low frequency and number of cases in human studies and limited data.
8007-00-9	Balsams, Peru (<i>Myroxylon pereirae</i>)		OECD TG 429: EC3 > 2% (1 study).	–	General population studies frequency ≥ 0.2% (1/1 study – dependent on gender and year). Unselected dermatitis patients frequency ≥ 1% (17/17 studies). Selected dermatitis patients frequency ≥ 2% (2/3 studies). Workplace selected workers frequency ≥ 1% (0/1 studies). N _(cases) > 100.	1 Based on the high frequency and number of cases in human studies and borderline result from the LLNA. SCCS substance of special concern ^{d)} .
84787-70-2 / 8006-87-9	Sandalwood, ext. / Sandalwood oil		–	–	Unselected dermatitis patients frequency ≥ 1% (4/7 studies). Selected dermatitis patients frequency ≥ 2% (5/7 studies). N _(cases) < 100.	1 Based on the high frequency of cases in human studies. SCCS substance of special concern ^{d)} .

LLNA: Local Lymph Node Assay; **GPMT OECD TG:** Guinea Pig Maximisation Test OECD Test Guideline. **SCCS (2012):** Scientific Committee on Consumer Safety, Opinion on Fragrance allergens in cosmetic products (2012).

- a) Criteria for LLNA sub-categorisation 1A: EC₃ value ≤ 2%.
Criteria for LLNA sub-categorisation 1B: EC₃ value > 2%.
- b) Criteria for GPMT sub-categorisation 1A: ≥ 30% responding at ≤ 0.1% intradermal induction dose or ≥ 60% responding at > 0.1% to ≤ 1% intradermal induction dose.
Criteria for GPMT sub-categorisation 1B: ≥ 30% to < 60% responding at > 0.1% to ≤ 1% intradermal induction dose or ≥ 30% responding at > 1% intradermal induction dose.
Criteria for Buehler assay sub-categorisation 1A: ≥ 15% responding at ≤ 0.2% topical induction dose or ≥ 60% responding at > 0.2% to ≤ 20% topical induction dose.
Criteria for Buehler assay sub-categorisation 1B: ≥ 15% to < 60% responding at > 0.2% to ≤ 20% topical induction dose or ≥ 15% responding at > 20% topical induction dose.
- c) Human diagnostic patch test data: General population studies: ≥ 0.2% = high frequency; < 0.2% = low/moderate frequency.
Human diagnostic patch test data: Dermatitis patients (unselected, consecutive): ≥ 1% = high frequency; < 1% = low/moderate frequency.
Human diagnostic patch test data: Selected dermatitis patients: ≥ 2% = high frequency; < 2% = low/moderate frequency.
Human diagnostic patch test data: Work place studies: selected workers with known exposure or dermatitis: ≥ 1% = high frequency; < 1% = low/moderate frequency.
Human diagnostic patch test data: Work place studies: all or randomly selected workers: ≥ 0.4% = high frequency; < 0.4% = low/moderate frequency.
Human diagnostic patch test data: Number of published cases (N_(cases)) ≥ 100 cases = high frequency; < 100 cases = low/moderate frequency.
- d) Substances of special concern according to SCCS (2012) defined as more than 100 cases from human studies.

Appendix 4 Citral CAS RN 5392-40-5

Citral is the mixture of two isomers: cis-citral (neral) and trans-citral (geranial, i.e. the aldehyde of geraniol, which is a hapten by itself with a moderate sensitisation potency). Geranial and neral have been identified as metabolites of geraniol and have been both been identified as secondary oxidation products when geraniol autoxidises (SCCS, 2012).

Non-human information

Table 1 summarises relevant animal studies with citral i.e. Local Lymph Node Assays (LLNAs), Guinea Pig Maximization tests (GPMTs) and Buehler tests.

Table 1. Animal studies with citral.

Method	Results	Remarks/Comments	Reference
LLNA (OECD TG 429): 5, 10 and 25% citral. Vehicle: Acetone:Olive oil (AOO).	Citral was shown to have an EC3 value of 12.6%.		Basketter (2012).
LLNA: LLNA:BrdU-FCM. 5, 10 and 25% citral. Vehicle: AOO.	Citral was shown to have an EC3 value of 9.2%.	Citral was classified as a moderate skin sensitizer by Jung (2012).	Jung (2012).
LLNA: 0.4, 2, 4, 8 and 20% citral. Vehicle: 1:3 ethanol:diethyl phthalate (EtOH:DEP).	Citral was shown to have an EC3 value of 1.2% (0.079 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2004b) cited from SCCS (2012).
LLNA: 0.3, 1, 3, 10 and 30% citral. Vehicle: 0.1% α-tocopherol in 3:1 EtOH:DEP.	Citral was shown to have an EC3 value of 1.5% (0.099 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003k) cited from SCCS (2012).
LLNA: 0.3, 1, 3, 10 and 30% citral. Vehicle: 0.3% antioxidant mix (equal parts butylated hydroxytoluene (BHT), tocopherol and eugenol) in 3:1 EtOH:DEP.	Citral was shown to have an EC3 value of 2.1% (0.14 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003l) cited from SCCS (2012).
LLNA: 0.3, 1, 3, 10 and 30% citral. Vehicle: 0.1% Trolox C in 3:1 EtOH:DEP.	Citral was shown to have an EC3 value of 3.7% (0.24 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003m) cited from SCCS (2012).
LLNA: 0.3, 1, 3, 10 and 30% citral. Vehicle: 3:1 EtOH:DEP.	Citral was shown to have an EC3 value of 4.6% (0.3 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003n) cited from SCCS (2012).
LLNA: 0.3, 1, 3, 10 and 30% citral.	Citral was shown to have an EC3 value of	According to SCCS (2012) there were no	Unpublished summary report by RIFM 2009

Method	Results	Remarks/Comments	Reference
Vehicle: 0.3% antioxidant mix (equal parts BHT, tocopherol and eugenol) in 3:1 EtOH:DEP.	4.6% (0.3 M).	reported deviations from OECD TG 429.	(RIFM 2003o) cited from SCCS (2012).
LLNA: 0.3, 1, 3, 10 and 30% citral. Vehicle: 3:1 EtOH:DEP.	Citral was shown to have an EC3 value of 5.3% (0.35 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003p) cited from SCCS (2012).
LLNA: 0.3, 1, 3, 10 and 30% citral. Vehicle: 0.1% Trolox C in 3:1 EtOH:DEP.	Citral was shown to have an EC3 value of 5.8% (0.38 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003q) cited from SCCS (2012).
LLNA: 2.5, 5, 10, 25 and 50% citral. Vehicle: 1:3 EtOH:DEP.	Citral was shown to have an EC3 value of 6.3% (0.41 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003r) cited from SCCS (2012).
LLNA: 0.3, 1, 3, 10 and 30% citral. Vehicle: 0.1% α -tocopherol in 3:1 EtOH:DEP.	Citral was shown to have an EC3 value of 6.8% (0.44 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003s) cited from SCCS (2012).
LLNA: (concentration not reported) citral. Vehicle: AOO.	Citral was shown to have an EC3 value of 13%.		Basketter et al., 2002a cited from Lalko and Api (2008).
LLNA: 5, 10 and 25% citral. Vehicle: 4:1 AOO.	Citral was shown to have an EC3 value between 7 and 15%.	According to REACH- RD (2015c) the study was reliable with restrictions (reliability 2) and performed equivalent or similar to OECD TG 429.	Basketter and Scholes 1992 cited from REACH- RD (2015c).
GPMT: Intradermal induction 0.1% citral; Topical induction 5% citral; Challenge dose 0.5%. Vehicle not reported.	6/10 (60%) animals were positive.	According to REACH- RD (2015d) the study is reliable with restrictions (reliability 2) and performed equivalent or similar to OECD TG 406.	Basketter and Allenby 1991; Basketter et al., 1991 and Basketter & Scholes, 1992 cited from REACH-RD (2015d).
GPMT: Intradermal induction 10% citral; Topical induction 10% citral; Challenge dose 10%. Vehicle not reported.	Sensitization observed.	No further information is available from Lalko and Api (2008).	Ishihara et al., 1986a cited from Lalko and Api (2008).
GPMT: Intradermal induction 0.4% citral;	Sensitization observed.	No further information is available from Lalko and Api (2008).	Goodwin and Johnson 1985 cited from Lalko and Api (2008).

Method	Results	Remarks/Comments	Reference
Topical induction 1% citral; Challenge dose 0.25%. Vehicle not reported.			
GPMT: Intradermal induction 25% citral in paraffin oil DAB7 or Freund's adjuvant/aqua dest. (1:1); Topical induction 25% citral in paraffin oil DAB7; Challenge doses 10, 5 and 5% citral in paraffin oil DAB7.	100% positive reactions.	According to REACH-RD (2015e) the study is reliable with restrictions (reliability 2) and performed equivalent or similar to OECD TG 406.	Study report 1978-11-15 cited from REACH-RD (2015e).
GPMT: Intradermal induction 25% citral in paraffin oil DAB7 or Freund's adjuvant/aqua dest. (1:1); Topical induction 25% citral in paraffin oil DAB7; Challenge doses 10, 5 and 5% citral in paraffin oil DAB7.	100% positive reactions except for after 144 hours after a 5% rechallenge where it was 60%.	According to REACH-RD (2015f) the study is reliable with restrictions (reliability 2) and performed equivalent or similar to OECD TG 406.	Study report 1978-11-12 cited from REACH-RD (2015f).
GPMT: Intradermal induction 5% citral; Topical induction 25% citral in pet.; Challenge dose: subirritant. Vehicle not reported.	Sensitization observed.	No further information is available from Lalko and Api (2008).	Klecak et al., 1977 cited from Lalko and Api (2008).
Buehler: Induction concentration 20% citral in petrolatum (pet.); Challenge dose 20% in pet.	Sensitization observed.	No further information is available from Lalko and Api (2008).	Unpublished report by RIFM 1973 cited from Lalko and Api (2008).

A total of 14 LLNAs, 6 GPMTs and 1 Buehler test are summarised in table 1. The reported EC3 values for citral range between 1.2% (vehicle: 1:3 ethanol:diethyl phthalate) and 15% (vehicle: Acetone:Olive oil 4:1). In the GPMTs sensitisation was observed but not quantified (i.e. number of animals affected) in 3/6 studies with intradermal induction doses of 0.4, 5 and 10% citral. In the other GPMTs 60% of the animals responded after an intradermal induction dose of 0.1% while 60-100% of the animals responded after an intradermal induction dose of 25% citral. Sensitisation was also observed but not quantified (i.e. number of animals affected) in the Buehler test with an induction concentration of 20% citral.

No relevant *in vitro* studies on citral (i.e. OECD TG 442C and OECD TG 442D) were identified in the literature.

Human information

Population studies

Table 2 summarises patch test studies on citral involving several thousand dermatitis patients from various countries in Europe and Asia. Most of the studies are diagnostic patch test studies.

Table 2. Population studies with citral.

Method	Results	Remarks/Comments	Reference
Patch test: Retrospective study of 324 selected patients patch tested with citral 2% in petrolatum (pet.).	42/324 (13%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2007-2009).	Schnuch et al. (2015).
Patch test: Prospective study of 655 consecutive patients patch tested with citral 3.5% in pet.	6/655 (0.92%) patients were positive.	A prospective study of patch test data at the Department of Dermatology, Sahlgrenska University Hospital, Gothenburg, Sweden (2010-2011).	Hagvall and Brared Christensson (2014).
Patch test: Retrospective study of 1951 selected eczema patients patch tested with citral 2% in pet.	20/1951 (1%, 95% CI: 0.6-1.4%) patients were positive.	A retrospective study on patch test data at St John's Institute of Dermatology at St Thomas' Hospital, UK (2011-2012).	Mann et al. (2014).
Patch test: Prospective study of 1055 consecutive patients patch tested with citral 1.5% in pet.	7/1055 (0.66%) patients were positive.	A prospective study on patch test data from Department of Dermatology, Sahlgrenska University Hospital, Gothenburg, Sweden (2006-2010).	Hagvall et al. (2012).
Patch test: Prospective study of 565 selected patients patch tested with citral 2% in vaseline.	19/565 (3.4%) patients were positive.	A prospective study on patch test data from multicentre study, Hungary (2009-2010).	Ponyai et al. (2012).
Patch test: Retrospective study of 205 selected patients tested with citral 2% in pet.	23/205 (11.2%) patients were positive.	A retrospective study on patch test data from Department of Dermatology, University Hospital St Rafaël, Belgium (1990-2011).	Nardelli et al. (2013).
Patch test: Prospective study	9/100 (9%, 95% CI: 4.2-16.4%)	Single-centre, double-blind prospective	Nagtegaal et al. (2012).

Method	Results	Remarks/Comments	Reference
of 100 selected patients with contact allergy patch tested with citral 2% in pet.	patients were positive.	experimental longitudinal volunteer study at the department of Dermatology of the VU University Medical Centre, The Netherlands (2005-2010).	
Patch test: Retrospective study of 1502 consecutive eczema patients patch tested with citral 2% in pet.	5/1502 (0.3 %) patients were positive.	A retrospective study of patch test data at Department of Dermato-Allergology, Copenhagen University Hospital Gentofte, Denmark (2008-2010).	Heisterberg et al. (2011, 2012).
Patch test: Prospective study of 30 selected patients (with positive reactions to ascaridole 1 and 5 %) patch tested with citral 2% in pet.	2/30 (7%) patients were positive.	A prospective study of patch test data at the Department of Dermatology, University Medical Centre Groningen, The Netherlands (2008-2011).	Bakker et al. (2011).
Patch test: Retrospective study of 86 selected patients patch tested with citral 2% in pet.	2/86 (2.3%) patients were positive.	A retrospective and descriptive analysis of a patch test study at the Cutaneous Allergy Unit of a tertiary referral hospital, Spain (2004-2008).	Cuesta et al. (2010).
Patch test: Retrospective study of 367 selected fragrance mix (FM) II positive patients patch tested with citral 2% in pet.	59/367 (16.1%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (2005-2008).	Krautheim et al. (2010).
Patch test: Prospective study of 320 selected eczema patients patch tested with citral 2% in pet.	2/320 (0.6%) patients were positive.	A prospective analysis of selected eczema patients at the University Medical Center in Groningen, the Netherlands (2005-2007).	van Oosten et al. (2009).
Patch test: Retrospective study of 2021 consecutive	12/2021 (0.6%, 95% CI: 0.3-1.1%) patients were	A retrospective study on patch test data from multicentre	Schnuch et al. (2007).

Method	Results	Remarks/Comments	Reference
patients patch tested with citral 2% in pet.	positive.	project IVDK (2003-2004).	
Patch test: Prospective study of 422 selected patients with suspected contact allergy patch tested with citral 2% in pet.	5/422 (1.2%) patients were positive.	A prospective analysis of patients from nine dermatology departments of university hospitals in Korea (2002-2003).	An et al. (2005).
Patch test: Prospective study of 1701 consecutive patients patch tested with citral 2% in pet.	12/1701 (0.7%) patients were positive.	A prospective analysis of patients from six dermatology departments (Dortmund, Copenhagen, Malmö, Odense, London and Leuven) (1997-1998).	Frosch et al., 2005 cited from SCCS (2012).
Patch test: Study of 1701 patients patch tested with citral 1%. Vehicle not reported.	6/1701 (0.35%) patients were positive.		Frosch et al., 2004 and 2005 cited from Lalko and Api (2008).
Patch test: Study of 586 consecutive patients patch tested with citral 2% in pet.	28/586 (4.8%) patients were positive.	According to SCCS 2012 irritant reactions were observed in 82/586 (14%).	Heydorn et al., 2003 cited from SCCS (2012).
Patch test: Prospective study of 1855 consecutive patients patch tested with citral 2% in pet.	21/1855 (1.1%) patients were positive.	A prospective analysis of patients from six dermatology departments (Dortmund, Copenhagen, Malmö, Odense, London and Leuven) (1997-1998).	Frosch et al. (2002a).
Patch test: Prospective study of 1825 consecutive patients patch tested with citral 2% in pet.	19/1825 (1%) patients were positive.	Multicenter study of patch test data in The Netherlands (1998-1999).	de Groot et al. (2000).
Patch test: Study of 192 patients patch tested with citral 1% in pet.	8/192 (4.2%) patients were positive.		Frosch et al., 1995 cited from Lalko and Api (2008).
Patch test: Study of	1/192 (0.5%)	According to Lalko and	Frosch et al.,

Method	Results	Remarks/Comments	Reference
192 patients patch tested with citral 0.1%. Vehicle not reported.	patients were positive.	Api (2008) the reaction was questionable.	1995 cited from Lalko and Api (2008).
Patch test: Study of 78 selected patients sensitive to FM patch tested with citral 2% in pet.	13/78 (16.7%) patients were positive.	Multicentre study involving 6 countries. Year not stated.	Wilkinson et al., 1989 cited from SCCNFP (1999).
Patch test: Study of 310 cosmetic dermatitis patients; 408 non-cosmetic patients and 122 control subjects patch tested with citral in 5%. Vehicle not reported.	8/310 (2.6%) cosmetic dermatitis patients; 9/408 (2.2%) non-cosmetic patients and 1/122 (0.8%) control subjects were positive.		Itoh et al., 1986 and 1988 and Nishimura et al., 1984 cited from Lalko and Api (2008).
Patch test: Study of 240 cosmetic dermatitis patients; 584 non-cosmetic patients and 105 control subjects patch tested with citral 2%. Vehicle not reported.	1/240 (0.4%) cosmetic dermatitis patients; 2/584 (0.3%) non-cosmetic patients and 0/105 (0%) control subjects were positive.		Itoh et al., 1986 and 1988 and Nishimura et al., 1984 cited from Lalko and Api (2008).
Patch test: Prospective study of 182 selected patients suspected of contact allergy to cosmetics patch tested with citral 2% in pet.	5/182 (2.6%) patients were positive.		Malten et al., 1984 cited from SCCNFP (1999).
Patch test: Study of 228 patients patch tested with citral 1% in pet.	4/228 (1.7%) patients were positive.	North American Contact Dermatitis Research Group (1973-1974).	Michell et al., 1982 cited from SCCNFP (1999).
Patch test: Study of 155 cosmetic dermatitis patients and 159 eczema/dermatitis patients patch tested with citral 5% in pet.	4/155 (2.6%) cosmetic dermatitis patients and 5/159 (3.1%) eczema/dermatitis patients were positive.	According to Lalko and Api (2008) a total of 48 control subjects were also tested with citral (5% pet) with no positive reactions.	Ishihara et al., 1981 cited from Lalko and Api (2008).
Patch test:	1/4 (25%) patients		Malten 1979

Method	Results	Remarks/Comments	Reference
Occupational study of 4 bakers with hand eczema patch tested with citral 0.5% in pet.	were positive.		cited from SCCNFP (1999).

Table 3 summarises Human Repeat Insult Patch Tests (HRIPTs) and Human Maximisation Tests (HMTs) with citral.

Table 3. HRIPT and HMT studies with citral adapted from Lalko and Api (2008).

Method	Results	Remarks/Comments	Reference
HRIPT: Citral concentration: 1.2%. Vehicle: 3:1 ethanol:diethyl phthalate (EtOH:DEP) with 0.2% tocopherol.	2/101 (2%) tests were positive.	According to REACH-RD (2015g) the study is reliable with restrictions (reliability 2) and performed according to HRL Protocol #100RIFM and HRL Standard Operating Procedures.	Study report 2004-09-11 cited from REACH-RD (2015g).
HRIPT: Citral concentration: 1.2% (1400 $\mu\text{g}/\text{cm}^2$). Vehicle: 1:3 EtOH:DEP.	0/101 (0%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 2004b cited from Lalko and Api (2008).
HRIPT: Citral concentration: 4- 8%. Vehicle: not reported.	19/40 (48%) tests were positive.		Opdyke 1979 cited from SCCFNP (1999).
HRIPT: Citral concentration: 4% (1240 $\mu\text{g}/\text{cm}^2$). Vehicle: petrolatum (pet.).	0/50 (0%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1971a cited from Lalko and Api (2008).
HRIPT: Citral concentration: 1% (775 $\mu\text{g}/\text{cm}^2$). Vehicle: alcohol SDA39C.	0/40 (0%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1965 cited from Lalko and Api (2008).

Method	Results	Remarks/Comments	Reference
HRIPT: Citral concentration: 5% (3876 µg/cm ²). Vehicle: alcohol SDA39C.	5/8 (62.5%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1964a cited from Lalko and Api (2008).
HRIPT: Citral concentration: 0.5% (388 µg/cm ²). Vehicle: alcohol SDA39C.	0/41 (0%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1964b cited from Lalko and Api (2008).
HMT: Citral concentration: 5% (3448 µg/cm ²). Vehicle: pet.	16/25 (64%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1974a cited from Lalko and Api (2008).
HMT: Citral concentration: 5% (3448 µg/cm ²). Vehicle: pet.	14/25 (56%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1974c cited from Lalko and Api (2008).
HMT: Citral concentration: 5% (3448 µg/cm ²). Vehicle: pet.	12/25 (48%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1974c cited from Lalko and Api (2008).
HMT: Citral concentration: 5% (3448 µg/cm ²). Vehicle: pet.	8/25 (32%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1974c cited from Lalko and Api (2008).
HMT: Citral concentration: 5% (3448 µg/cm ²). Vehicle: pet.	11/24 (45.8%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1974d cited from Lalko and Api (2008).
HMT: Citral	0/25 (0%) tests were positive.	No further information available from Lalko	Unpublished report from RIFM

Method	Results	Remarks/Comments	Reference
concentration: 5% (3448 µg/cm ²). Vehicle: butylene glycol.		and Api (2008).	1974e cited from Lalko and Api (2008).
HMT: Citral concentration: 4% (2759 µg/cm ²) Vehicle: pet.	3/25 (12%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1972b cited from Lalko and Api (2008).
HMT: Citral concentration: 4% (2759 µg/cm ²). Vehicle: pet.	3/25 (12%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1972c cited from Lalko and Api (2008).
HMT: Citral concentration: 4% (2759 µg/cm ²). Vehicle: pet.	5/25 (20%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1972c cited from Lalko and Api (2008).
HMT: Citral concentration: 2% (1379 µg/cm ²). Vehicle: pet.	2/24 (8.3%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1972d cited from Lalko and Api (2008).
HMT: Citral concentration: 8% (5517 µg/cm ²) Vehicle: pet.	8/24 (33.3%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1971b cited from Lalko and Api (2008).
HMT: Citral concentration: 4% (2759 µg/cm ²). Vehicle: pet.	9/25 (36%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1971c cited from Lalko and Api (2008).
HMT: Citral concentration: 4% (2759 µg/cm ²). Vehicle: pet.	4/25 (16%) tests were positive.	No further information available from Lalko and Api (2008).	Unpublished report from RIFM 1971c cited from Lalko and Api (2008).
HMT:	5/25 (20%)	No further information	Unpublished

Method	Results	Remarks/Comments	Reference
Citral concentration: 4% (2759 µg/cm ²). Vehicle: pet.	tests were positive.	available from Lalko and Api (2008).	report from RIFM 1971c cited from Lalko and Api (2008).

HRIPT: Human Repeat Insult Patch Test, HMT: Human Maximisation Test.

Case studies

Table 4 summarises case reports with ACD where citral has been found to be among the causative agents.

Table 4. Case studies with citral.

Method	Results	Remarks/Comments	Reference
Patch test: A 30-year old female patient with recurrent allergic contact cheilitis was patch tested with fragrance mix (FM) II and citral.	Strong positive reaction to FM II and citral. The cheilitis was attributed to a lip salve containing citral.	Case study (year not reported).	Hindle et al., 2007 cited from SCCS (2012).
Patch test: Over a period of 2 years a total of 9 beauticians with bilateral hand dermatitis were patch tested with the British baseline series, FM I and II, cosmetics and own products.	Positive reactions in 5 of the 9 beauticians were observed.	Multiple case study (UK).	De Mozzi and Johnston (2014).

A total of 30 results from patch test population studies, 7 HRIPTs, 14 HMTs and 2 case studies with citral are summarised above (Table 2, 3 and 4). As shown in Table 2 the positive patch test frequencies from all of the reported patch test population studies vary between 0.3 and 16.7% in dermatitis patients. For unselected/consecutive dermatitis patients, positive reactions range between 0.3 and 4.8% (8 studies) and for selected dermatitis patients positive reactions range between 0.3 and 16.7% (22 studies). The total number of published cases is > 300. Sensitisation was reported in 3/7 HRIPT studies at a citral concentration of 3876 µg/cm² (5%). In the HMT studies 13/14 studies with citral showed a positive result after 1379 µg/cm². Based on these data the Research Institute for Fragrance Materials, Inc. (RIFM) deducted a NOEL-HRIPT⁴ (induction) of 1400 µg/cm² and a LOEL-HRIPT/HMT⁵ (induction) of 3876 µg/cm². In addition, based on weight of evidence, a No Expected Sensitization Induction Level (NESIL) of 1400 µg/cm² was established for citral by the RIFM Expert Panel (IFRA, 2013a).

⁴ NOEL-HRIPT: No Observed Effect Level-Human Repeat Insult Patch Test

⁵ LOEL-HRIPT/HMT: Lowest Observed Effect Level- Human Repeat Insult Patch Test/Human Maximisation test

Citral is a "top 100" substance and has a harmonised classification for skin sensitisation (SCCS, 2012; DK-EPA, 2015).

According to SCCS (2012) citral is used in volumes greater than 175 ton per year in perfume formulations. It has been reported that in consumer products containing fragrance allergens that are required to be labelled 11.6% of a total of 516 consumer products; 25% of a total of 300 consumer products; ca. 12% of 3000 products and 8.2% of children's cosmetics were labelled to contain citral (Wijnhoven et al., 2008; Buckley, 2007; Schnuch et al., 2009 and Poulsen & Schmidt, 2007 cited from SCCS (2012)). In addition, in 2007, 26.1% of 88 tested deodorants were labelled to contain citral and the fragrance was detected in 44% (range: 39-554 mg/kg) of 23 deodorants selected for analysis (Rastogi et al., 2007 cited from SCCS (2012)).

The IFRA standard limits for citral in different IFRA QRA product categories reported by IFRA (2013a and 2015) are shown in table 5.

Table 5. The IFRA standard limits for citral in IFRA QRA product categories.

IFRA QRA product category	Product type that drives the category consumer exposure level	IFRA standard limits
Category 1	Lip products	0.04%
Category 2	Deodorants/antiperspirants	0.05%
Category 3	Hydroalcohols for shaved skin	0.2%
Category 4	Hydroalcohols for unshaved skin	0.6%
Category 5	Hand cream	0.3%
Category 6	Mouthwash	1.0%
Category 7	Intimate wipes	0.1%
Category 8	Hair styling aids	1.4%
Category 9	Rinse-off hair conditioners	5.0%*
Category 10	Hard surface cleaners	2.5%*
Category 11	Candles	Not restricted

IFRA: International Fragrance Association, QRA: Quantitative Risk Assessment.

*Maximum pragmatic level.

Citral is registered under the REACH regulation with an annual tonnage band of 1000 - 10 000 tonnes per annum.

Summary and discussion of skin sensitization

Human data

A total of 30 results from patch test population studies, 7 HRIPTs, 14 HMTs and 2 case studies were identified for citral. The positive patch test frequencies from all of the reported patch test population studies vary between 0.3 and 16.7% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0.3 and 4.8% (8 studies) and in studies with selected dermatitis patients positive reactions range between 0.3 and 16.7% (22 studies). The total number of published cases is > 300. Sensitisation was reported in 3/7 HRIPT studies after exposure to 3876 µg/cm² (5%) and in 13/14 HMT studies after exposure to 1379 µg/cm².

Non-human data

A total of 12 LLNAs, 6 GPMTs and 1 Buehler test were identified testing skin sensitising effects of citral. The reported EC3 values for citral ranged between 1.2% and 15% in different vehicles. In the GPMTs sensitisation was observed but not quantified (i.e. number of animals affected) in 3/6 studies with intradermal induction doses of 0.4, 5 and 10% citral. In the other GPMTs sensitisation was observed in 60% of the animals after an intradermal induction dose of 0.1% and in 60-100% of the animals after an intradermal induction dose of 25% citral. Sensitisation was also observed but not quantified (i.e. number of animals affected) in the Buehler test with an induction concentration of 20% citral.

No relevant *in vitro* studies on citral (i.e. OECD TG 442C and OECD TG 442D) were identified in the literature.

Exposure

According to data from IFRA (2013a) the exposure of citral when used as fragrance in cosmetics and in other consumer products appears to be low.

Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 0.3 and 4.8% with 3/8 studies reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 0.3 and 16.7% with 14 out of 22 studies reporting frequencies higher than 2%. In addition to this there are more than 300 published cases of positive patch test reactions to citral. According to the CLP criteria a frequency $\geq 1\%$ for unselected/consecutive dermatitis patients and/or $\geq 2\%$ for selected dermatitis patients and/or a total number of published cases ≥ 100 , equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that citral causes a *high frequency* of occurrence of skin sensitisation based on patch test data from a minority of unselected dermatitis patient studies and a majority of selected dermatitis patient studies and the number of published cases. In regard to the HRIPT/HMT data the positive response reported at $> 500 \mu\text{g}/\text{cm}^2$ for HRIPT/HMT induction threshold indicate evidence for sub-category 1B according to Annex I: 3.4.2.2.2.2.

In the 14 LLNAs EC3 values between 1.2 and 15% were reported for citral. Two out of the 14 LLNAs reported an EC3 value $< 2\%$. According to the CLP Regulation an EC3 value $\leq 2\%$ indicates classification of a substance in sub-category 1A whereas an EC3 value $> 2\%$ indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.). Thus, these two studies indicate classification of citral in sub-category 1A. However, in the other 12 LLNAs, the EC3 value was $> 2\%$ with only one of these 12 LLNAs reporting an EC3 value (2.1%) borderline to the cut-off criteria for classification in sub-category 1A or 1B indicating classification of citral in sub-category 1B. Based on a weight of evidence for the LLNAs, classification of citral in sub-category 1B seems justified.

In 1/6 of the GPMTs sensitisation was observed in 60% of the animals after an intradermal induction dose of 0.1%. According to the CLP criteria a positive response $\geq 30\%$ of the animals responding at $\leq 0.1\%$ intradermal induction dose indicates classification of a substance in sub-category 1A (Annex I: 3.4.2.2.3.2.) and thus, this study indicates classification of citral into sub-category 1A. In 2/6 GPMTs sensitisation was observed in 60-100% of the animals after an intradermal induction dose of 25% citral. According to the CLP criteria a positive response $\geq 30\%$ responding at $> 1\%$ intradermal induction dose indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.); thus, these two studies indicate classification of citral in sub-category 1B. In 3/6 the GPMTs sensitisation was reported to be observed but not quantified (i.e. number of animals affected) with intradermal induction doses of 0.4, 5 and 10% citral; therefore, these GPMTs cannot be compared with the classification criteria.

Sensitisation was also reported to be observed but not quantified (i.e. number of animals affected) in the Buehler test with an induction concentration of 20% citral; therefore, this study cannot be compared with the classification criteria.

Overall, there is clear evidence for classification in sub-category 1A based on the human patch test studies showing a high frequency of occurrence of skin sensitisation and the total number of cases combined with the estimated low exposure. The results from the animal studies are equivocal, mainly indicating classification in sub-category 1B. A classification as a skin sensitizer in sub-category 1A is thus warranted for citral.

Conclusions on classification and labelling

Based on the high frequency of sensitisation observed in human patch test studies and the high number of published cases combined with the estimated low exposure, a classification of citral as a skin sensitizer in sub-category 1A is justified.

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Appendix 5 Cinnamaldehyde CAS RN 104-55-2

Non-human information

Table 1 summarises relevant animal studies with cinnamaldehyde i.e. Local Lymph Node Assays (LLNAs), Guinea Pig Maximization tests (GPMTs) and Buehler tests.

Table 1. Animal studies with cinnamaldehyde.

Method	Results	Remarks/Comments	Reference
LLNA: BrdU-ELISA <i>in vivo</i> and <i>ex vivo</i> BrdU. 1, 5 and 10% cinnamaldehyde.	Cinnamaldehyde was shown to have an EC2 value of 6.1% in the BrdU-ELISA <i>in vivo</i> and an EC2 value of 6.9 in the <i>ex vivo</i> BrdU.	Cinnamaldehyde was classified as positive for skin sensitisation by Williams et al. (2015).	Williams et al. (2015).
LLNA: 0.1, 0.99, 3.3, 9.9 and 19.8% cinnamaldehyde.	Cinnamaldehyde was shown to have an EC3 value of 0.75% (57 mM).	Cinnamaldehyde was classified as a strong skin sensitizer by Niklasson et al. (2013).	Niklasson et al. (2013).
LLNA: <i>Ex vivo</i> BrdU. 0.5, 1, 5 and 10% cinnamaldehyde. Vehicle: Acetone: Olive oil (AOO).	Cinnamaldehyde was shown to have an EC3 value of 1.91%.	Cinnamaldehyde was classified as a moderate skin sensitizer by Ulker et al. (2013).	Ulker et al. (2013).
LLNA: LLNA:BrdU-ELISA. 1, 3 and 10%. Vehicle: AOO.	Cinnamaldehyde was shown to have an EC2 value of 2.2%.	Cinnamaldehyde was classified as positive for skin sensitisation by Kojima (2011).	Kojima (2011).
LLNA: 0.1, 0.3, 1, 3 and 10% cinnamaldehyde. Vehicle: 3:1 ethanol:diethyl phthalate (EtOH:DEP).	Cinnamaldehyde was shown to have an EC3 value of 0.2% (0.015 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003a) cited from SCCS (2012).
LLNA: 0.1, 0.3, 1, 3 and 10% cinnamaldehyde. Vehicle: 0.1% α- tocopherol in 3:1 EtOH:DEP.	Cinnamaldehyde was shown to have an EC3 value of 0.2% (0.015 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003b) cited from SCCS (2012).
LLNA: 0.1, 0.3, 1, 3 and 10% cinnamaldehyde. Vehicle: 2% α-	Cinnamaldehyde was shown to have an EC3 value of 0.6%	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM

Method	Results	Remarks/Comments	Reference
tocopherol in 3:1 EtOH:DEP.	(0.045 M).		2003c) cited from SCCS (2012).
LLNA: 0.1, 0.3, 1, 3 and 10% cinnamaldehyde. Vehicle: 0.3% antioxidant mix (equal parts Butylated hydroxytoluene (BHT), tocopherol and eugenol) in 3:1 EtOH:DEP.	Cinnamaldehyde was shown to have an EC3 value of 0.7% (0.053 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003d) cited from SCCS (2012).
LLNA: 0.1, 0.3, 1, 3 and 10% cinnamaldehyde. Vehicle: 0.1% Trolox C in 3:1 EtOH:DEP.	Cinnamaldehyde was shown to have an EC3 value of 0.7% (0.053 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003e) cited from SCCS (2012).
LLNA: 0.1, 0.3, 1, 3 and 10% cinnamaldehyde. Vehicle: 2% α-tocopherol in 3:1 EtOH:DEP.	Cinnamaldehyde was shown to have an EC3 value of 0.8% (0.06 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003f) cited from SCCS (2012).
LLNA: 0.1, 0.3, 1, 3 and 10% cinnamaldehyde. Vehicle: 3:1 EtOH:DEP.	Cinnamaldehyde was shown to have an EC3 value of 0.9% (0.068 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003g) cited from SCCS (2012).
LLNA: 0.1, 0.3, 1, 3 and 10% cinnamaldehyde. Vehicle: 0.1% α-tocopherol in 3:1 EtOH:DEP.	Cinnamaldehyde was shown to have an EC3 value of 1.1% (0.083 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003h) cited from SCCS (2012).
LLNA: 0.1, 0.3, 1, 3 and 10% cinnamaldehyde. Vehicle: 0.3% antioxidant mix (equal parts BHT, tocopherol and	Cinnamaldehyde was shown to have an EC3 value of 1.3% (0.098 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003i) cited from SCCS (2012).

Method	Results	Remarks/Comments	Reference
eugenol) in 3:1 EtOH:DEP.			
LLNA: 0.1, 0.3, 1, 3 and 10% cinnamaldehyde. Vehicle: 0.1% Trolox C in 3:1 EtOH:DEP.	Cinnamaldehyde was shown to have an EC3 value of 1.4% (0.11 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003j) cited from SCCS (2012).
LLNA: concentration in vehicle not reported. Vehicle: 4:1 AOO.	Cinnamaldehyde was shown to have an EC3 value of 1.3% (0.10 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Elahi et al 2004 cited from SCCS (2012).
LLNA: 0.5, 1, 2.5, 5 and 10% cinnamaldehyde. Vehicle: 4:1 AOO.	Cinnamaldehyde was shown to have an EC3 value of 3.1% (0.23 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Basketter et al., 2001 cited from SCCS (2012).
LLNA: 1 and 2.5% cinnamaldehyde. Vehicle: 4:1 AOO.	Cinnamaldehyde was shown to have an EC3 value of 1.4% (0.11 M).	Too few concentrations tested and few details in reference according to SCCS (2012).	Smith and Hotchkiss 2001 cited from SCCS (2012).
LLNA: 1, 2.5, 5, 10 and 25% cinnamaldehyde. Vehicle: 50:50 EtOH:water.	Cinnamaldehyde was shown to have an EC3 value of 1.2% (0.091 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Wright et al., 2001 cited from SCCS (2012).
LLNA: 1, 2.5, 5, 10 and 25% cinnamaldehyde. Vehicle: 90:10 EtOH:water.	Cinnamaldehyde was shown to have an EC3 value of 1.6% (0.12 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Wright et al., 2000 cited from SCCS (2012).
LLNA: 0.1, 0.25, 0.5, 1, 2.5, 5, 10 and 25% cinnamaldehyde. Vehicle: Dimethyl sulfoxide (DMSO).	Cinnamaldehyde was shown to have an EC3 value of 0.9% (0.068 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Wright et al., 1999 cited from SCCS (2012).
LLNA: 1, 2.5, 5, 10 and 25% cinnamaldehyde. Vehicle: propylene glycol.	Cinnamaldehyde was shown to have an EC3 value of 1.4% (0.11 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Wright et al., 1998 cited from SCCS (2012).
LLNA: 0.1, 0.25, 0.5, 1, 2.5, 5, 10 and	Cinnamaldehyde was shown to	According to SCCS (2012) there were no	Wright et al., 1997 cited

Method	Results	Remarks/Comments	Reference
25% cinnamaldehyde. Vehicle: Dimethylformamide (DMF).	have an EC3 value of 0.5% (0.038 M).	reported deviations from OECD TG 429.	from SCCS (2012).
LLNA: 1, 2.5, 5, 10 and 25% cinnamaldehyde. Vehicle: Methyl ethyl ketone (MEK).	Cinnamaldehyde was shown to have an EC3 value of 1.1% (0.083 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Wright et al 1996 cited from SCCS (2012).
LLNA: 1, 2.5, 5, 10 and 25% cinnamaldehyde. Vehicle: 4:1 AOO.	Cinnamaldehyde was shown to have an EC3 value of 1.7% (0.13 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Wright et al., 1995 cited from SCCS (2012).
GPMT: Concentration: 0.75% cinnamaldehyde (2 samples). Vehicle: not reported.	9/10 (90%) and 10/10 (100%) animals were positive.		Basketter 1992 cited from Bickers et al. (2005).
GPMT: Concentration: 0.75% cinnamaldehyde. Vehicle: 70:30 acetone/polyethylene glycol (PEG) 400.	100% of the animals were sensitised.		Basketter and Scholes 1992 cited from Bickers et al. (2005).
GPMT: Concentration: 3% cinnamaldehyde. Vehicle: not reported.	Strong sensitisation effects reported (no further details).		Ishihara et al., 1986 cited from Bickers et al. (2005).

A total of 22 LLNAs, 2 LLNA BrdU-ELISA tests and 3 GPMTs are summarised in table 1. The reported EC3 values for cinnamaldehyde range between 0.2% (vehicle: 3:1 ethanol:diethyl phthalate with or without α -tocopherol) and 3.1% (vehicle: Acetone:Olive oil 4:1). In the LLNA BrdU-ELISA tests EC2 values were reported to be between 2.2 and 6.9%. Positive reactions were observed in all GPMTs with cinnamaldehyde concentrations down to 0.75%. It is, however, not clear from the review by Bickers et al. (2005) whether the concentration was the intradermal induction dose.

No relevant *in vitro* studies on cinnamaldehyde (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

Human information

Population studies

Table 2 summarises patch test studies on cinnamaldehyde involving several thousand dermatitis patients from various countries in Europe and the US. Most of the studies are diagnostic patch test studies.

Table 2. Population studies with cinnamaldehyde

Method	Results	Remarks/Comments	Reference
Patch test: Retrospective study of 806 selected patients patch tested with cinnamaldehyde 1% in petrolatum (pet.).	76/806 (9.4%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2007-2009).	Schnuch et al. (2015).
Patch test: Retrospective study of 1951 selected eczema patients patch tested with cinnamaldehyde 1% in pet.	27/1951 (1.4%, 95% CI: 0.9-1.9%) patients were positive.	A retrospective study on patch test data at St John's Institute of Dermatology at St Thomas' Hospital, UK (2011-2012).	Mann et al. (2014).
Patch test: Retrospective study of 41 selected children age 0-5 years; 838 selected children age 6-18 years and selected adults > 18 years patch tested with cinnamaldehyde 1% in pet.	2/41 (4.9%) children age 0-5 years; 10/838 (1.2%) children age 6-18 years and 516/17213 (3%) adults >18 years were positive.	A retrospective study of pooled patch test data from patients collected by the North American Contact Dermatitis Group (NACDG) (2005-2012).	Zug et al. (2014).
Patch test: Retrospective study of selected allergic contact dermatitis (ACD) patients patch tested with cinnamaldehyde 1% in pet.	122/5079 (2.4%) patients were positive.	A retrospective study of pooled patch test data from patients collected by the North American Contact Dermatitis Group (NACDG) (2007-2008).	Fransway et al. (2013).
Patch test: Retrospective study of 940 selected patients tested with cinnamaldehyde 1% in pet.	66/940 (7%) patients were positive.	A retrospective study on patch test data from Department of Dermatology, University Hospital St Rafaël, Belgium (1990-2011).	Nardelli et al. (2013).
Patch test: Retrospective study of 164 hairdressers and hairdressing apprentices with eczema were tested	3/164 (1%) patients were positive.	A retrospective study of patch test data at Department of Occupational Dermatology Research and Education Centre,	Lyons et al. (2013).

Method	Results	Remarks/Comments	Reference
with cinnamaldehyde 1% in pet.		Australia (1993-2010).	
Patch test: Prospective study of 23 selected patients with chronic idiopathic urticarial patch tested with cinnamaldehyde 1% in pet.	3/23 (13%) patients were positive.	A prospective longitudinal study at Tufts Medical Center, USA. Year not stated.	Hession and Scheinman (2012).
Patch test: Prospective study of 100 selected patients with contact allergy patch tested with cinnamaldehyde 1% in pet.	10/100 (10%, 95% CI: 4.9-17.62%) patients were positive.	Single-centre, double-blind prospective experimental longitudinal volunteer study at the department of Dermatology of the VU University Medical Centre, The Netherlands (2005-2010).	Nagtegaal et al. (2012).
Patch test: Retrospective study of 1503 consecutive eczema patients patch tested with cinnamaldehyde 1% in pet.	20/1503 (1.3%, 95% CI: 0.8-2%) patients were positive.	A retrospective study of patch test data at Department of Dermato-Allergology, Copenhagen University Hospital Gentofte, Denmark (2008-2010).	Heisterberg et al. (2011).
Patch test: Retrospective study of 157 selected patients (chosen out of 509 patients positive to fragrance allergens) patch tested with cinnamaldehyde 1% pet.	Ca. 24/157 (ca. 15%) patients were positive.	A retrospective study of patch test data at the Allergy Clinic of the Department of Dermatology and Venereology, Zagreb University Hospital Center and School of Medicine, Zagreb, Croatia (2001-2005).	Turcic et al. (2011).
Patch test: Retrospective study of 1214 consecutive patients and 4527 selected patients patch tested with cinnamaldehyde in 1% pet.	17/1214 (1.43%, 95% CI: 0.67-2.18%) and 120/4527 (2.64%, 95% CI: 2.16-3.13%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2005-2008).	Uter et al. (2010).
Patch test: Retrospective study	7/86 (8.1%) patients were	A retrospective and descriptive analysis of	Cuesta et al. (2010).

Method	Results	Remarks/Comments	Reference
of 86 selected patients patch tested with cinnamaldehyde 2% in pet.	positive.	a patch test study at the Cutaneous Allergy Unit of a tertiary referral hospital, Spain (2004-2008).	
Patch test: Retrospective study of 774 consecutive eczema patients patch tested with cinnamaldehyde 1% in pet.	66/774 (8.5%) patients were positive.	Retrospective study of patch test data from Odense University Hospital, Denmark (1995-2007).	Andersen et al. (2009).
Patch test: Prospective study of 18 selected cinnamon-sensitive patients patch tested with cinnamaldehyde 2% in pet.	4/18 (22%) patients were positive.	Prospective study of cinnamon-sensitive patients at the Department of Dermatology of the VU University Medical Centre, The Netherlands (year not stated).	Pentling et al. (2009).
Patch test: Prospective study of 320 selected eczema patients patch tested with cinnamaldehyde 1% in pet.	5/320 (1.6%) patients were positive.	A prospective analysis of selected eczema patients at the University Medical Center in Groningen, the Netherlands (2005-2007).	van Oosten et al. (2009).
Patch test: Retrospective study of 364 selected patients with a) current allergic dermatitis or b) past allergic dermatitis patch tested with cinnamaldehyde 1% in pet.	a) 38/364 (10.4%) and b) 67/364 (19.2%) patients were positive.	A retrospective study of patch test data from patients attending the Department of Cutaneous Allergy at St John's Institute of Dermatology, UK (1982-2007).	White (2009).
Patch test: Retrospective study of selected ACD patients patch tested with cinnamaldehyde 1% in pet. between year 2003-2004: 5138 patients and year 2005-2006: 4435 patients.	Year 2003-2004: 123/5138 (2.4%) and year 2005-2006: 138/4435 (3.1%) patients were positive.	A retrospective study of pooled patch test data from patients collected by the North American Contact Dermatitis Group (NACDG) (2005-2006).	Zug et al. (2009).

Method	Results	Remarks/Comments	Reference
Patch test: Prospective study of 15 selected patients with eczematous reactions from ketoprofen-containing gels patch tested with cinnamaldehyde 1% in pet.	1/15 (6.7%) patients were positive.	A prospective study on patch test data from patients from Italy (2006-2007).	Foti et al. (2008).
Patch test: Retrospective study of selected ACD patients patch tested with cinnamaldehyde 1% in pet. over two decades. Year 1984-1985: 1199 patients; year 1985-1989: 3964 patients; year 1992-1994: 3528 patients; year 1994-1996: 3112 patients; year 1996-1998: 3443 patients and year 1998-2000: 4735 patients.	Year 1984-1985: 71/1199 (5.9%); year 1985-1989: 123/3964 (3.1%); year 1992-1994: 95/3528 (2.7%); year 1994-1996: 75/3112 (2.4%); year 1996-1998: 96/3443 (2.8%) and year 1998-2000: 170/4735 (3.7%) patients were positive.	A retrospective study of pooled patch test data from patients collected by the North American Contact Dermatitis Group (NACDG) (1970-2002).	Nguyen et al. (2008).
Patch test: Retrospective study of 2063 unselected patients patch tested with cinnamaldehyde 1% in pet.	21/2063 (1%, 95% CI: 0.5-1.5%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (2003-2004).	Schnuch et al. (2007).
Patch test: Prospective study of 1603 selected patients with eczematous dermatitis patch tested with cinnamaldehyde 1% in pet.	27/1603 (1.7%) patients were positive.	A prospective analysis of multicentre data on patients from five US sites and one Canadian site (year not reported)	Belsito et al. (2006).
Patch test: Study of 30 selected patients with a positive patch test to their own	6/30 (20%) patients were positive.		Vocanson et al. (2006).

Method	Results	Remarks/Comments	Reference
perfumed product patch tested with cinnamaldehyde. Concentration and vehicle not reported.			
Patch test: Prospective study of 422 selected patients with suspected contact allergy patch tested with cinnamaldehyde 1% in pet.	7/422 (1.7%) patients were positive.	A prospective analysis of patients from nine dermatology departments of university hospitals in Korea (2002-2003).	An et al. (2005).
Patch test: Retrospective study of 4900 unselected patients patch tested with cinnamaldehyde 1% in pet.	93/4900 (1.9%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (1996-1999).	Schnuch et al. (2002).
Patch test: Prospective study of 747 selected patients with suspected fragrance allergy patch tested with cinnamaldehyde 1% in pet.	14/747 (1.9%) patients were positive.	A prospective analysis of patients from FAZ- Floridsdorf Allergy Centre, Austria (1997- 2000).	Wohrl et al. (2001).
Patch test: Study of 226 selected patients sensitive to FM patch tested with cinnamaldehyde 1% in pet.	30/226 (13%) patients were positive.	Department of Dermatology, University Hospital, Coimbra, Portugal (1989-1999)	Brites et al. (2000).
Patch test: Retrospective study of 50 patients sensitive to FM patch tested with cinnamaldehyde 2% in 1% sorbitan sesquioleate.	10/50 (20%) patients were positive.	Retrospective study of patch test data. University Hospital Utrecht, The Netherlands (1994- 1998).	Hendriks and van Ginkel (1999).
Patch test: Retrospective study of 40 patients sensitive to FM patch tested with	5/40 (12.5%) patients were positive.		Katsarma and Gawkrodger (1999).

Method	Results	Remarks/Comments	Reference
cinnamyl alcohol in pet. Concentration not reported.			
Patch test: Study of 167 selected patients suspected of fragrance sensitivity patch tested with cinnamaldehyde 1% in pet.	24/167 (14.4%) patients were positive.		Larsen et al., 1996 cited from SCCNFP (1999).
Patch test: Prospective study of 1072 consecutive patients patch tested with cinnamaldehyde 1% in pet.	10/1072 (0.93%) patients were positive.	Prospective study of patients in a multicentre study involving 9 European centres. Year not stated.	Frosch et al., 1995 cited from SCCNFP (1999).
Patch test: Retrospective study of 2447 consecutive patients from three age groups patch tested between 1979-1983 with 2% pet. and 3440 consecutive patients from three age groups patch tested between 1988-1992 with cinnamaldehyde 1% in pet.	Between 1979 and 1983 754-795/2447 (30.8-32.5%) and between 1988 and 1992 313-440/3440 (9.1-12.8%) patients were positive.	Retrospective study of patch test data from Department of Dermatology, Gentofte Hospital, Denmark (1979-1983 and 1988-1992).	Johansen and Menne (1995).
Patch test: Prospective study of 61 selected patients sensitive to FM patch tested with cinnamaldehyde 2% in pet.	21/61 (34%) patients were positive. Control tests in 100 patients not allergic to fragrances showed no positive reactions when tested with cinnamaldehyde 2% pet.	Prospective study of patch test data from University of Amsterdam and University of Leiden, The Netherlands (1987).	de Groot et al. (1993).
Patch test: Prospective study of 162 selected patients positive to a	34/162 (21%) patients were positive.	Retrospective study of patch test data from Dermatologische Klinik und Poliklinik, Germany	Enders et al., 1989 cited from SCCNFP (1999).

Method	Results	Remarks/Comments	Reference
fragrance mix patch tested with cinnamaldehyde 1%. Vehicle not reported.		(1987).	
Patch test: Study of 78 selected patients positive to a fragrance mix patch tested with cinnamaldehyde 1%. Vehicle not reported.	10/78 (12.8%) patients were positive.	Multicentre study involving 6 countries. Year not stated.	Wilkinson et al., 1989 cited from SCCNFP (1999).
Patch test: Retrospective study of 1200 selected patients with dermatitis patch tested between 1983 and 1984 with cinnamaldehyde 2% in pet. and 1500 selected patients with dermatitis patch tested between 1984 and 1985 with cinnamaldehyde 1% in pet.	Between 1983 and 1984 9/63 (14.3%) and between 1984 and 1985 3/54 (5.6%) patients were positive.	Retrospective study of patch test data from Istituto Dermatologico Santa Maria e San Gallicano, Italy (1983-1985).	Santucci et al. (1987).
Patch test: Retrospective study of 403 selected patients with cutaneous reactions to cosmetic products patch tested with cinnamaldehyde. Concentration and vehicle not reported.	6/403 (1.5%) patients were positive.	It is unclear from the reference exactly how many patients were tested with cinnamyl alcohol.	Adams and Maibach (1985).
Patch test: Prospective study of 182 selected patients suspected of contact allergy to cosmetics patch tested with cinnamaldehyde 0.5% in pet.	7/182 (3.7%) patients were positive.		Malten et al., 1984 cited from SCCNFP (1999).
Patch test: Study of 20 selected perfume allergic patients	6/20 (30%) patients were positive.		Larsen et al., 1977 cited from SCCNFP

Method	Results	Remarks/Comments	Reference
patch tested with cinnamaldehyde 1%. Vehicle not reported.			(1999).
Patch test and ROAT ¹ : 17 cinnamaldehyde-allergic patients (20 controls) were tested with a dilution series of cinnamaldehyde in a patch test and a ROAT.	No threshold could be established as all tested doses gave positive reactions in the ROAT. Minimum tested dose was 0.26 µg/cm ² .	Copenhagen, Denmark and Malmö, Sweden. Year not stated.	Bruze et al. (2003).
Patch test and ROAT ¹ : 22 cinnamaldehyde-allergic patients (20 controls) were tested with a dilution series of cinnamaldehyde in a patch test and a ROAT.	The ROAT threshold in percentage was higher than the patch test threshold.	Clinical study at Gentofte Hospital and Odense University Hospital, Denmark. Year not stated.	Johansen et al. (1996).

¹ROAT: Repeat Open Application Test.

Table 3 summarises Human Repeat Insult Patch Tests (HRIPTs) and Human Maximisation Tests (HMTs) with cinnamaldehyde.

Table 3. HRIPT and HMT studies with cinnamaldehyde adapted from Bickers et al. (2005).

Method	Results	Remarks/Comments	Reference
HRIPT: Cinnamaldehyde concentration: 0.5% Vehicle: 3:1 diethyl phthalate:ethanol (DEP:EtOH).	0/94 (0%) tests were positive.		Unpublished report (RIFM 2004) cited from Bickers et al. (2005).
HRIPT: Cinnamaldehyde concentration: 3% Vehicle: 3:1 DEP:EtOH with 0.5% α-tocopherol.	4/28 (14%) tests were positive.		Unpublished report (RIFM 2003b) cited from Bickers et al. (2005).
HRIPT: Cinnamaldehyde concentration: 3%	Study aborted during induction phase due to the		Unpublished report (RIFM 2003b) cited

Method	Results	Remarks/Comments	Reference
Vehicle: 3:1 DEP:EtOH with 0.5% α-tocopherol.	number or irritant reactions.		from Bickers et al. (2005).
HRIPT: Cinnamaldehyde concentration: 0.5% Vehicle: 3:1 DEP:EtOH with 0.5% α-tocopherol.	0/22 (0%) tests were positive.		Unpublished report (RIFM 2002a) cited from Bickers et al. (2005).
HRIPT: Cinnamaldehyde concentration: 0.5% Vehicle: 3:1 DEP:EtOH with 0.5% α-tocopherol.	0/19 (0%) tests were positive.		Unpublished report (RIFM 2002b) cited from Bickers et al. (2005).
HRIPT: Cinnamaldehyde concentration: 0.1, 0.5, 1 or 1.25% Vehicle: EtOH.	0/41 (0%), 0/38 (0%), 5/41 (12%) and 5/10 (50%) were positive after 0.1, 0.5, 1 or 1.25% cinnamaldehyde, respectively.		Danneman et al., 1983 cited from Cocchiara et al. (2005).
HRIPT: Cinnamaldehyde concentration: 1% Vehicle: EtOH or petrolatum (pet.).	1/55 (2%) tests were positive with ethanol as vehicle, no reactions with petrolatum as vehicle.		Marzulli and Maibach 1976 and 1980 cited from Bickers et al. (2005).
HRIPT: Cinnamaldehyde concentration: 1% Vehicle: alcohol SDA 39C.	5/41 (12%) tests were positive.		Unpublished report (RIFM 1973d) cited from Bickers et al. (2005).
HRIPT: Cinnamaldehyde concentration: 0.5% Vehicle: EtOH.	0/38 (0%) tests were positive.		Unpublished report (RIFM 1965) cited from Bickers et al. (2005).
HRIPT: Cinnamaldehyde concentration:	0/41 (0%) tests were positive.		Unpublished report (RIFM 1964a) cited

Method	Results	Remarks/Comments	Reference
0.125% Vehicle: EtOH.			from Bickers et al. (2005).
HRIPT: Cinnamaldehyde concentration: 1.25% Vehicle: EtOH.	5/10 (50%) tests were positive.		Unpublished report (RIFM 1964b) cited from Bickers et al. (2005).
HMT: Cinnamaldehyde concentration: 3% Vehicle: butylene glycol.	3/25 (12%) tests were positive.		Unpublished report (RIFM 1974a) cited from Bickers et al. (2005).
HMT: Cinnamaldehyde concentration: 2% Vehicle: pet.	11/25 (44%) tests were positive.		Unpublished report (RIFM 1973c) cited from Bickers et al. (2005).

HRIPT: Human Repeat Insult Patch Test, HMT: Human Maximisation Tests.

Case studies

Table 4 summarises case reports with allergic contact dermatitis in different clinics in Europe and the US where cinnamaldehyde has been found as a causative agent.

Table 4. Case studies with cinnamaldehyde

Method	Results	Remarks/Comments	Reference
Patch test: A 33-year old man with itching eczematous lesions was patch tested with cinnamaldehyde. Concentration and vehicle not reported.	Positive reaction on day 2 and day 4 was observed.	Case study, Italy (year not stated).	Guarneri (2010).
Patch test: A 47-year old man with dermatitis was patch tested with cinnamaldehyde. Concentration and vehicle not reported.	Positive reaction on day 2 was observed.	Case study, USA (year not stated).	Decapite and Anderson (2004).
Patch test: A 42-year old woman with rash on her arms was patch tested with cinnamaldehyde. Concentration and vehicle not reported.	Positive reaction after 20 min (anaphylaxis) was observed.	Case study, UK (year not stated).	Diba and Statham (2003).

A total of 52 results from patch test population studies, 2 repeat open application tests (ROATs), 10 HRIPTs, 2 HMTs and 3 case studies with cinnamaldehyde are summarised above (Table 2, 3 and 4). As shown in Table 2 the positive patch test frequencies from all of the reported patch test population studies vary between 0.93 and 38% in dermatitis patients. For unselected/consecutive dermatitis patients, positive reactions range between 0.93 and 32.5% (8 studies) and for selected dermatitis patients positive reactions range between 1.2 and 90% (44 studies). A single study in workers reported positive patch test reactions in 1%. The total number of published cases is > 2300. Sensitisation was reported in 6/12 HRIPT studies at cinnamaldehyde concentrations between 1 and 3%. Both HMT studies reported positive reactions after 2-3% cinnamaldehyde. The Research Institute for Fragrance Materials, Inc. (RIFM) deducted a NOEL-HRIPT⁶ (induction) of 591 µg/cm² and a LOEL-HRIPT/HMT⁷ (induction) of 775 µg/cm². In addition, based on weight of evidence, a No Expected Sensitization Induction Level (NESIL) of 590 µg/cm² was established for cinnamaldehyde by the RIFM Expert Panel (IFRA, 2013b).

⁶ NOEL-HRIPT: No Observed Effect Level-Human Repeat Insult Patch Test

⁷ LOEL-HRIPT/HMT: Lowest Observed Effect Level- Human Repeat Insult Patch Test/ Human Maximisation test

Two ROATs with cinnamaldehyde are summarised in table 2 (Johansen et al., 1996; Bruze et al., 2003). Results from these studies may be used for establishing a specific concentration limit for cinnamaldehyde in consumer products.

According to SCCS (2012) cinnamaldehyde is used in volumes less than 175 ton per year in perfume formulations. It has been reported that 2.5% of a total of 516 consumer products; 6% of a total of 300 fragrance products; ca. 2% of 3000 products and 1% of children cosmetics were labelled to contain cinnamaldehyde (Wijnhoven et al., 2008; Buckley, 2007; Schnuch et al., 2009 and Poulsen & Schmidt, 2007 cited from SCCS (2012)). In addition, in 2007, 1.1% of 88 tested deodorants were labelled to contain cinnamaldehyde and the fragrance was detected in 4% (range: 5 mg/kg) of 23 deodorants selected for analysis (Rastogi et al., 2007 cited from SCCS (2012)). Besides exposure from use of cosmetic products, cinnamaldehyde exposure also occurs from clothing, candles and food (SCCS, 2012).

The IFRA standard limits for cinnamaldehyde in different IFRA QRA product categories reported by IFRA (2013b and 2015) are shown in table 5.

Table 5. The IFRA standard limits for cinnamaldehyde in IFRA QRA product categories.

IFRA QRA product category	Product type that drives the consumer exposure level	IFRA standard limits
Category 1	Lip products	0.02 %
Category 2	Deodorants/antiperspirants	0.02 %
Category 3	Hydroalcohols for shaved skin	0.05 %
Category 4	Hydroalcohols for unshaved skin	0.05 %
Category 5	Hand cream	0.05 %
Category 6	Mouthwash	0.4 %
Category 7	Intimate wipes	0.04 %
Category 8	Hair styling aids	0.05 %
Category 9	Rinse-off hair conditioners	0.05 %
Category 10	Hard surface cleaners	0.05 %
Category 11	Candles	Not restricted

IFRA: International Fragrance Association, QRA: Quantitative Risk Assessment.

Cinnamaldehyde is registered under the REACH regulation with an annual tonnage band of 1000 - 10 000 tonnes per annum.

Summary and discussion of skin sensitization

Human data

A total of 52 results from patch test population studies, 7 HRIPTs, 14 HMTs and 2 case studies were identified with cinnamaldehyde. The positive patch test frequencies from all of the reported patch test population studies vary between 0.93 and 90% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0.93 and 32.5% (8 studies) and in studies with selected dermatitis patients positive reactions range between 1.2 and 90% (44 studies). A single study in workers reported positive patch test reactions in 1%. The total number of published cases is > 2300. A LOEL-HRIPT/HMT (induction) of 775 µg/cm² was established for cinnamaldehyde by the RIFM Expert Panel based on unpublished reports.

Non-human data

A total of 22 LLNAs and 2 LLNA BrdU-ELISA tests were identified testing skin sensitising effects of cinnamaldehyde. The reported EC3 values for cinnamaldehyde ranged between 0.2% and 3.1% in different vehicles.

No relevant *in vitro* studies on cinnamaldehyde (i.e. OECD TG 442C and OECD TG 442D) were identified in the literature.

Exposure

According to IFRA (2013b) the exposure of cinnamaldehyde when used as fragrance in cosmetics and in other consumer products appears to be low.

Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 0.93 and 32.5% with 7/8 studies reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 1.2 and 90% with 37 out of 44 studies reporting frequencies higher than 2%. A single study in workers reported positive patch test reactions in 1%. In addition to this there are more than 2300 published cases of positive patch test reactions to cinnamaldehyde. According to the CLP criteria a frequency $\geq 1\%$ for unselected/consecutive dermatitis patients and/or $\geq 2\%$ for selected dermatitis patients and/or $\geq 1\%$ for selected workers with known exposure or dermatitis and/or a total number of published cases ≥ 100 , equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that cinnamaldehyde causes a *high frequency* of occurrence of skin sensitisation based on these four types of information.

In regard to the HRIPT/HMT data the positive response reported at $> 500 \mu\text{g}/\text{cm}^2$ for HRIPT/HMT induction threshold indicate evidence for sub-category 1B according to Annex I: 3.4.2.2.2.2.

In the 22 LLNAs EC3 values between 0.2 and 3.1% were reported for cinnamaldehyde with 21/22 EC3 values $< 2\%$. According to the CLP Regulation an EC3 value $\leq 2\%$ indicates classification of a substance in sub-category 1A (Annex I: 3.4.2.2.3.2.). Hence, data from LLNAs indicate classification of cinnamaldehyde in sub-category 1A.

Overall, there is clear evidence for classification in sub-category 1A based on the human patch test studies showing a high frequency of occurrence of skin sensitisation and the total number of cases combined with the estimated low exposure. Data from HRIPT/HMT indicate evidence for sub-category 1B. Data from the LLNAs support a sub-category 1A classification. A classification as a skin sensitiser in sub-category 1A is thus warranted for cinnamaldehyde.

Conclusions on classification and labelling

Based on the high frequency of sensitisation observed in human patch test studies and the high number of published cases combined with the estimated low exposure and supported by data from LLNAs, a classification of cinnamaldehyde as a skin sensitiser in sub-category 1A is justified.

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Appendix 6 Cinnamyl alcohol CAS RN 104-54-1

According to SCCS (2012) cinnamyl alcohol is a fragrance compound known to be a prohapten and to form sensitising compounds by metabolic transformation, which increases the likelihood for cross-reactivity between cinnamyl alcohol and cinnamaldehyde.

Non-human information

Table 1 summarises relevant animal studies with cinnamyl alcohol i.e. Local Lymph Node Assays (LLNAs), Guinea Pig Maximization tests (GPMTs) and Buehler tests.

Table 1. Animal studies with cinnamyl alcohol.

Method	Results	Remarks/Comments	Reference
LLNA: <i>Ex vivo</i> LLNA-BrdU ELISA. 0, 0.5, 1, 5 and 10% cinnamyl alcohol. Vehicle: Acetone: olive oil (AOO).	Cinnamyl alcohol was shown to have an EC3 value of 17.9%.	Cinnamyl alcohol was classified as a weak skin sensitizer by Ulker et al. (2014).	Ulker et al. (2014).
LLNA (OECD TG 429): 10, 25 and 50% cinnamyl alcohol. Vehicle: AOO	Cinnamyl alcohol was shown to have an EC3 value of 25.2%.		Basketter (2012).
LLNA: LLNA:BrdU-FCM. 10, 25 and 50% cinnamyl alcohol. Vehicle: 4:1 AOO.	Cinnamyl alcohol was shown to have an EC3 value of 21%.	Cinnamyl alcohol was classified as positive for skin sensitisation by Jung (2012).	Jung (2012).
LLNA (OECD TG 429): Cinnamyl alcohol (concentration not reported). Vehicle: 4:1 AOO.	Cinnamyl alcohol was shown to have an EC3 value of 20.1% (1.5 M).		Elahi et al. (2004).
LLNA: 10, 25, 50 and 90% cinnamyl alcohol. Vehicle: 4:1 AOO.	Cinnamyl alcohol was shown to have an EC3 value of 21%.	Cinnamyl alcohol was classified as a weak skin sensitizer by Gerberick et al. (2005).	Estrada et al., 2003 cited from Gerberick et al. (2005).
GPMT: Intradermal induction 25%; topical induction 25%; challenge dose 3% cinnamyl alcohol. Vehicle: not reported.	Positive reactions in 3/10 (30%) of the tested animals.		Modjtahedi (2011).

Method	Results	Remarks/Comments	Reference
GPMT: Concentration: 10% cinnamyl alcohol. Vehicle: not reported.	Strong sensitisation effects reported (no further details).		Ishihara et al., 1986 cited from Bickers et al. (2005).
Freund's complete adjuvant test: Intradermal induction dose 5% with challenge doses of 3, 10, 30 and 100% cinnamyl alcohol. Vehicle: not reported.	Positive reactions in 0/20, 0/20, 0/20 and 3/20 (15%) animals, respectively.		Study report 1986-01-06 cited from REACH-RD (2015k).
Buehler test: Induction dose 3, 10 and 30% with a challenge dose of 3% cinnamyl alcohol. Vehicle: not reported.	Positive reactions in 0/11, 0/13 and 0/15 animals, respectively.		Modjtahedi (2011).

A total of five LLNAs (two of which were reported to be conducted in accordance with OECD TG 429), two GPMTs, one Freund's complete adjuvant test (FCAT) and one Buehler test were identified testing the skin sensitisation of cinnamyl alcohol. EC3 values between 17.9 and 30% were reported for cinnamyl alcohol in the LLNAs. Sensitisation was observed in 30% of the animals in a GPMT after an intradermal induction dose of 25%. Another GPMT reported strong sensitisation effects but without specifying the number of affected animals. In the Freund's complete adjuvant test 15% of the animals had positive reactions after an intradermal induction dose of 100% cinnamyl alcohol. No positive reactions were observed in the Buehler test after induction doses of 3, 10 and 30% cinnamyl alcohol.

No relevant *in vitro* studies on cinnamyl alcohol (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

Human information

Population studies

Table 2 summarises patch test studies on cinnamyl alcohol involving several thousand dermatitis patients from various countries in Europe and Asia. Most of the studies are diagnostic patch test studies.

Table 2. Population studies with cinnamyl alcohol.

Method	Results	Remarks/Comments	Reference
Patch test: Retrospective study of 806 selected patients	66/806 (8.2%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information	Schnuch et al. (2015).

Method	Results	Remarks/Comments	Reference
patch tested with cinnamyl alcohol 1% in petrolatum (pet.).		Network of Departments of Dermatology) (2007-2009).	
Patch test: Retrospective study of 1951 selected eczema patients patch tested with cinnamyl alcohol 2% in pet.	48/1951 (2.5%, 95% CI: 1.8-3.1%) patients were positive.	A retrospective study on patch test data at St John's Institute of Dermatology at St Thomas' Hospital, UK (2011-2012).	Mann et al. (2014).
Patch test: Retrospective study of 940 selected patients tested with cinnamyl alcohol 1% in pet.	129/940 (13.7%) patients were positive.	A retrospective study on patch test data from Department of Dermatology, University Hospital St Rafaël, Belgium (1990-2011).	Nardelli et al. (2013).
Patch test: Prospective study of 100 selected patients with contact allergy patch tested with cinnamyl alcohol 1% in pet.	13/100 (13%, 95% CI: 7.11-21.20%) patients were positive.	Single-centre, double-blind prospective experimental longitudinal volunteer study at the department of Dermatology of the VU University Medical Centre, The Netherlands (2005-2010).	Nagtegaal et al. (2012).
Patch test: Retrospective study of 1501 consecutive eczema patients patch tested with cinnamyl alcohol 1% in pet.	10/1501 (0.7%, 95% CI: 0.3-1.9%) patients were positive.	A retrospective study of patch test data at Department of Dermato-Allergology, Copenhagen University Hospital Gentofte, Denmark (2008-2010).	Heisterberg et al. (2011).
Patch test: Retrospective study of 157 selected patients (chosen out of 509 patients positive to fragrance allergens) patch tested with cinnamyl alcohol 5% in pet.	54/157 (34.4%) patients were positive.	A retrospective study of patch test data at the Allergy Clinic of the Department of Dermatology and Venereology, Zagreb University Hospital Center and School of Medicine, Zagreb, Croatia (2001-2005).	Turcic et al. (2011).

Method	Results	Remarks/Comments	Reference
Patch test: Retrospective study of 86 selected patients patch tested with cinnamyl alcohol 2% in pet.	12/86 (13.9%) patients were positive.	A retrospective and descriptive analysis of a patch test study at the Cutaneous Allergy Unit of a tertiary referral hospital, Spain (2004-2008).	Cuesta et al. (2010).
Patch test: Prospective study of 18 selected cinnamon-sensitive patients patch tested with cinnamyl alcohol 2% in pet.	5/18 (28%) patients were positive.	Prospective study of cinnamon-sensitive patients at the Department of Dermatology of the VU University Medical Centre, The Netherlands (year not stated).	Pentinga et al. (2009).
Patch test: Prospective study of 320 selected eczema patients patch tested with cinnamyl alcohol in 2% pet.	8/320 (2.5%) patients were positive.	A prospective analysis of selected eczema patients at the University Medical Center in Groningen, the Netherlands (2005-2007).	van Oosten et al. (2009).
Patch test: Retrospective study of 266 selected patients with a) current allergic dermatitis or b) past allergic dermatitis patch tested with cinnamyl alcohol 1% in pet.	a) 24/266 (9.02%) and b) 44/266 (16.54%) patients were positive.	A retrospective study of patch test data from patients attending the Department of Cutaneous Allergy at St John's Institute of Dermatology, UK (1982-2007).	White (2009).
Patch test: Prospective study of 15 selected patients with eczematous reactions from ketoprofen-containing gels patch tested with cinnamyl alcohol 2% in pet.	15/15 (100%) patients were positive.	A prospective study on patch test data from patients from Italy (2006-2007).	Foti et al. (2008).
Patch test: Retrospective study of 2063 unselected	13/2063 (0.6%, 95% CI: 0.2-1.0%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (2003-2004).	Schnuch et al. (2007).

Method	Results	Remarks/Comments	Reference
patients patch tested with cinnamyl alcohol 1% in pet.			
Patch test: Retrospective study of a) 29 patients positive to their own deodorant and b) 133 negative to their own deodorant patch tested with cinnamyl alcohol 1% in pet.	a) 3/29 (10%) and b) 2/133 (1.5%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (1998-2002).	Uter et al. (2007).
Patch test: Study of 30 selected patients with a positive patch test to their own perfumed product patch tested with cinnamyl alcohol. Concentration and vehicle not specified.	6/30 (20%) patients were positive.		Vocanson et al. (2006).
Patch test: Prospective study of 422 selected patients with suspected contact allergy patch tested with cinnamyl alcohol 2% in pet.	13/422 (3.1%) patients were positive.	A prospective analysis of patients from nine dermatology departments of university hospitals in Korea (2002-2003).	An et al. (2005).
Patch test: Retrospective study of 4900 unselected patients patch tested with cinnamyl alcohol 1% in pet.	88/4900 (1.8%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (1996-1999).	Schnuch et al. (2002).
Patch test: Prospective study of 747 selected patients with suspected	11/747 (1.5%) patients were positive.	A prospective analysis of patients from FAZ-Floridsdorf Allergy Centre, Austria (1997-2000).	Wohrl et al. (2001).

Method	Results	Remarks/Comments	Reference
fragrance allergy patch tested with cinnamyl alcohol 1% in pet.			
Patch test: Study of 226 selected patients sensitive to FM patch tested with cinnamyl alcohol 1% in pet.	18/226 (8%) patients were positive.	Department of Dermatology, University Hospital, Coimbra, Portugal (1989-1999)	Brites et al. (2000).
Patch test: Retrospective study of 50 patients sensitive to FM patch tested with cinnamyl alcohol 2% in 1% sorbitan sesquioleate.	8/50 (16%) patients were positive.	Retrospective study of patch test data. University Hospital Utrecht, The Netherlands (1994- 1998).	Hendriks and van Ginkel (1999).
Patch test: Retrospective study of 40 patients sensitive to FM patch tested with cinnamyl alcohol in pet. Concentration not reported.	4/40 (10%) patients were positive.		Katsarma and Gawkrodger (1999).
Patch test: Study of 167 selected patients suspected of fragrance sensitivity patch tested with cinnamyl alcohol 5% in lanolin.	11/167 (6.6%) patients were positive.		Larsen et al., 1996 cited from SCCNFP (1999).
Patch test: Prospective study of 1072 consecutive patients patch tested with cinnamyl alcohol 1% in pet.	6/1072 (0.56%) patients were positive.	Prospective study of patients in a multicentre study involving 9 European centres. Year not stated.	Frosch et al., 1995 cited from SCCNFP (1999).
Patch test: Retrospective study of 367 selected patients patch tested with	40/367 (10.9%) patients were positive.	Retrospective study of patch test data from Department of Dermatology, Gentofte Hospital, Denmark	Johansen and Menne (1995).

Method	Results	Remarks/Comments	Reference
cinnamyl alcohol 1-2% in pet.		(1979-1983 and 1988-1992).	
Patch test: Prospective study of 50 selected patients positive to a fragrance mix (FM) patch tested with cinnamyl alcohol. Concentration not reported.	17/50 (34%) patients were positive.	Retrospective study of patch test data from Department of Dermatology and Venereology, Hungary. Year not stated.	Becker et al. (1994).
Patch test: Prospective study of 61 selected patients sensitive to a FM patch tested with cinnamyl alcohol 5% in pet.	19/61 (31%) patients were positive. Control tests in 100 patients not allergic to fragrances showed that cinnamyl alcohol was marginally irritant at the concentration chosen.	Prospective study of patch test data from University of Amsterdam and University of Leiden, The Netherlands (1987).	de Groot et al. (1993).
Patch test: Study of selected patients positive to a FM patch tested with cinnamyl alcohol. Concentration and vehicle not reported.	14% patients were positive.	Study of patch test data from France. No further information available from SCCNFP 1999.	Artigou et al., 1989 cited from SCCNFP (1999).
Patch test: Prospective study of 162 selected patients positive to a FM patch tested with cinnamyl alcohol 1%. Vehicle not reported.	9/162 (5.6%) patients were positive.	Retrospective study of patch test data from Dermatologische Klinik und Poliklinik, Germany (1987).	Enders et al. (1989).
Patch test: Study of 78 selected patients positive to a FM patch tested with cinnamyl alcohol 1%.	5/78 (6.4%) patients were positive.	Multicentre study involving 6 countries. Year not stated.	Wilkinson et al., 1989 cited from SCCNFP (1999).

Method	Results	Remarks/Comments	Reference
Vehicle not reported.			
Patch test: Study of 119 selected patients with contact allergy to cosmetic products patch tested with cinnamyl alcohol 5% in pet.	2/119 (1.7%) patients were positive.		De Groot et al., 1988 cited from SCCNFP (1999).
Patch test: Study of 156 selected patients with pure contact allergy to cosmetic products patch tested with cinnamyl alcohol Concentration and vehicle not reported.	6/156 (3.8%) patients were positive.		Broneck et al., 1987 cited from SCCNFP (1999).
Patch test: Retrospective study of 63 selected patients with dermatitis tested positive to perfume mixture patch tested between 1983 and 1984 with cinnamyl alcohol 3% in pet. and 54 selected patients with dermatitis tested positive to perfume mixture patch tested between 1984 and 1985 with cinnamyl alcohol 1% in pet.	Between 1983 and 1984 9/63 (14.3%) and between 1984 and 1985 5/54 (9.3%) patients were positive.	Retrospective study of patch test data from Istituto Dermatologico Santa Maria e San Gallicano, Italy (1983-1985).	Santucci et al. (1987).
Patch test: Retrospective study of 403 selected patients with cutaneous reactions to cosmetic products patch tested with cinnamyl alcohol.	17/403 (4.2%) patients were positive.	It is unclear from the reference exactly how many patients were tested with cinnamyl alcohol.	Adams and Maibach (1985).

Method	Results	Remarks/Comments	Reference
Concentration and vehicle not reported.			
Patch test: study of 20 selected perfume allergic patients patch tested with cinnamyl alcohol 5%. Vehicle not reported.	15/20 (75%) patients were positive.		Larsen et al., 1977 cited from SCCNFP (1999).

Table 3 summarises Human Repeat Insult Patch Tests (HRIPTs) and Human Maximisation Tests (HMTs) with cinnamyl alcohol.

Table 3. HRIPT and HMT studies with cinnamyl alcohol adapted from Letizia et al. (2005).

Method	Results	Remarks/Comments	Reference
HRIPT: Sample: NA ¹ 4%. Vehicle: 1:3 ethanol:diethyl phthalate (EtOH:DEP).	2/54 (4%) at re-challenge, subjects reacted under occluded conditions but not under semi-occlusive conditions or in a 5-day repeated open application test.	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (2001a, 2002a) cited from Letizia et al. (2005).
HRIPT: Sample: NA 4%. Vehicle: 3:1 EtOH:DEP.	1/55, however as subject also reacted to vehicle control and to neat ethanol, it was concluded that the reaction was caused by the ethanol component and not cinnamyl alcohol	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (2001b, 2002b) cited from Letizia et al. (2005).
HRIPT (modified Draize): Sample: NA 4%. Vehicle: EtOH and petrolatum (pet.).	4/150 (2.7%) reactions with ethanol as vehicle; no reactions with petrolatum as vehicle.		Jordan and King 1977 cited from Letizia et al. (2005).
HMT:	4/28 (14%) were	No further information	Unpublished

Method	Results	Remarks/Comments	Reference
Sample: 82-10-17 (prepared via a borohydride reduction process) 10%. Vehicle: DEP.	positive (virgin panel)	available from Letizia et al. (2005).	report from RIFM (1982a) cited from Letizia et al. (2005).
HMT: Sample: 82-10-654 10%. Vehicle: DEP.	4/27 questionable para-allergic reactions; 3 of these subjects were retested and 2/3 reacted	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1982b) cited from Letizia et al. (2005).
HMT: Sample: 81-10-HR recrystallized sample 10%. Vehicle: DEP.	2/22 (9%) were positive.	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1981a) cited from Letizia et al. (2005).
HMT: Sample: 81-10-HR (retest) 10%. Vehicle: DEP.	2/23 (8.7%) were positive (virgin panel)	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1981b) cited from Letizia et al. (2005).
HMT: Sample: 79-10-ED-UN 10%. Vehicle: DEP.	6/28 (21%) were positive.	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1980a) cited from Letizia et al. (2005).
HMT: Sample: 79-10-ED-W (washed with alkali) 10%. Vehicle: DEP.	0/24 (0%) were positive.	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1980b) cited from Letizia et al. (2005).
HMT: Sample: 79-10-0 10%. Vehicle: DEP.	2/22 (9%) were positive plus 1 questionable reaction.	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1980d) cited from Letizia et al. (2005).
HMT: Sample: 79-10-N (pure form of 79-10-0) 10%. Vehicle: DEP.	0/35 sensitisation reactions (0%); 7 irritations reactions and 1 hyper-irritation reaction.	No further information available from Letizia et al. (2005) and Bickers et al 2005.	Unpublished report from RIFM (1980e) cited from Letizia et al. (2005) and Bickers et al. (2005).
HMT: Sample: 79-10-ED-W and F	1/28 (4%) was positive.	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1980c) cited

Method	Results	Remarks/Comments	Reference
(washed with alkali) 10%. Vehicle: DEP.			from Letizia et al. (2005).
HMT: Sample: CA (washed with alkali) 10%. Vehicle: DEP	1/21 questionable reactions (virgin panel)	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1980f) cited from Letizia et al 2005
HMT: Sample: 79-10-CADEP 10%. Vehicle: DEP.	6/26 (23%) were positive.	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1979a) cited from Letizia et al. (2005).
HMT: Sample: 79-4-5J 4%. Vehicle: pet.	1 irritation reaction in 24 Japanese Americans	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1979b) cited from Letizia et al. (2005).
HMT: Sample: SKK-10-OX 10%. Vehicle: pet.	10/33 (30%) were positive plus 3 questionable and 2 irritant reactions	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1977a) cited from Letizia et al. (2005).
HMT: Sample: CA-10-STY (extracted from styrax) 10%. Vehicle: pet.	5/25 (20%) were positive	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1977b) cited from Letizia et al. (2005).
HMT: Sample: 76-Bedoukianol-10 (2 nd retest) 10%. Vehicle: pet.	3/25 (12%) were positive	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1977d) cited from Letizia et al. (2005).
HMT: Sample: SKK-10-P (pure form of SKK-10-OX) 10%. Vehicle: pet.	1/24 (4%) was positive plus 3 questionable and 2 irritant reactions.	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1977e) cited from Letizia et al. (2005).
HMT: Sample: 35-10-35R (0) (retest) 10%. Vehicle: hydrophilic ointment.	2/25 (8%) were positive	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1976a) cited from Letizia et al. (2005).
HMT:	7/25 (28%) were	No further information	Unpublished

Method	Results	Remarks/Comments	Reference
Sample: 76-Bedoukianol-10 10%. Vehicle: pet.	positive	available from Letizia et al. (2005).	report from RIFM (1976b) cited from Letizia et al. (2005).
HMT: Sample: 76-Bedoukianol-10 (retest) 10%. Vehicle: pet.	9/25 (36%) were positive	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1976c) cited from Letizia et al. (2005).
HMT: Sample: Cinnamyl alcohol 10%. Vehicle: hydrophilic ointment	2/25 (8%) were positive	No further information available from Bickers et al. (2005).	Unpublished report from RIFM (1976d) cited from Bickers et al. (2005).
HMT: Sample: CA 10%. Vehicle: pet.	5/25 (20%) were positive (virgin panel)	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1976e) cited from Letizia et al. (2005).
HMT: Sample: 76-10FDO-B 10%. Vehicle: pet.	1/11 (9%) were positive	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1976f) cited from Letizia et al. (2005).
HMT: Sample: 35-10-35R (0) 10%. Vehicle: pet.	3/25 (12%) were positive	No further information available from Letizia et al. (2005).	Unpublished report from RIFM (1975) cited from Letizia et al. (2005).
HMT: Sample: NA 4%. Vehicle not reported.	0/25 (0%) were positive.		Greif 1967 cited from Letizia et al. (2005).
Modified HMT: Sample: NA 4%. Vehicle: EtOH and pet.	1 reaction (25-30 subjects) with ethanol as vehicle; no reactions with petrolatum as vehicle.		Jordan and King 1977 cited from Letizia et al. (2005).

¹NA: Not Available

Case studies

Table 4 summarises case reports with allergic contact dermatitis in different clinics in Europe where cinnamyl alcohol has been found as a causative agent.

Table 4. Case studies with cinnamyl alcohol.

Method	Results	Remarks/Comments	Reference
Patch test: A 33-year old man with itching eczematous lesions was patch tested with cinnamyl alcohol. Concentration and vehicle not reported.	Positive reaction on Day 2 (D2) and D4 was observed.	Case study, Italy (year not stated).	Guarneri (2010).
Patch test: An 18-year old woman with acute eczema was patch tested with cinnamyl alcohol 5% in petrolatum (pet.).	Positive reaction on D2 and D3 was observed.	Case study, Italy (year not stated).	Lauriola et al. (2009).
Patch test: A 74-year old woman with extensive eczematous and bullous dermatitis was patch tested with cinnamyl alcohol 1% in pet.	Positive reaction on D2 and D4 was observed.	Case study, Spain (year not stated).	Garcia-Abujeta et al. (2005).
Patch test: A 47-year old man with vesicular dermatitis was patch tested with cinnamyl alcohol 1% in pet.	Positive reaction on D2 and D3 was observed.	Case study, Germany (year not stated).	Hartmann and Hunzelmann (2004).

A total of 34 patch test population studies, 3 HRIPTs, 25 HMTs and 4 case studies with cinnamyl alcohol are summarised above (Table 2, 3 and 4). As shown in Table 1 the positive patch test frequencies from all of the reported patch test studies vary between 0.56 and 100% in dermatitis patients. For unselected/consecutive dermatitis patients positive reactions range between 0.56 and 1.8% (4 studies) and for selected dermatitis patients positive reactions range between 1.5 and 100% (30 studies). The total number of published cases is > 600. Sensitisation was reported in 2/3 HRIPT studies at a cinnamyl alcohol concentration of 4%. In the HMT studies 17/24 studies with cinnamyl alcohol showed a positive result after 10%. The Research Institute for Fragrance materials, Inc. (RIFM) deducted a NOEL⁸-HRIPT (induction) of 3000 µg/cm², a NOEL-HMT (induction) of 2759 µg/cm² and a LOEL⁹ (induction) of 4724 µg/cm² for cinnamyl alcohol. In addition, based on weight of evidence, a No Expected Sensitization Induction Level (NESIL) of 3000 µg/cm² was established for cinnamyl alcohol by the RIFM Expert Panel (IFRA, 2008a).

⁸NOEL: No Observed Effect Level

⁹LOEL: Lowest Observed Effect Level

According to SCCS (2012) cinnamyl alcohol is used in volumes less than 175 ton per year in perfume formulations. It has been reported that 6.4% of a total of 516 consumer products; 8% of a total of 300 fragrance products, ca. 4% or 3000 products and 6.7% of children cosmetics were labelled to contain cinnamyl alcohol (Wijnhoven et al., 2008; Buckley, 2007; Schnuch et al., 2009 and Poulsen & Schmidt, 2007 cited from SCCS (2012)). In addition, in 2007, 12.5% of 88 tested deodorants were labelled to contain cinnamyl alcohol and the fragrance was detected in 48% (range: 2-503 mg/kg) of 23 deodorants selected for analysis (Rastogi et al., 2007 cited from SCCS (2012)).

The IFRA standard limits for cinnamyl alcohol in different IFRA QRA product categories reported by IFRA (2008a and 2015) are shown in table 5.

Table 5. The IFRA standard limits for cinnamyl alcohol in IFRA QRA product categories.

IFRA category	QRA product	Product type that drives the category consumer exposure level	IFRA standard limits
Category 1		Lip products	0.09%
Category 2		Deodorants/antiperspirants	0.1%
Category 3		Hydroalcohols for shaved skin	0.4%
Category 4		Hydroalcohols for unshaved skin	0.4%
Category 5		Hand cream	0.4%
Category 6		Mouthwash	2.2%
Category 7		Intimate wipes	0.2%
Category 8		Hair styling aids	0.4%
Category 9		Rinse-off hair conditioners	0.4%
Category 10		Hard surface cleaners	0.4%
Category 11		Candles	Not restricted

IFRA: International Fragrance Association, QRA: Quantitative Risk Assessment.

Cinnamyl alcohol is registered under the REACH regulation with an annual tonnage band of 100-1000 tonnes/year.

Summary and discussion of skin sensitization

Human data

A total of 34 patch test population studies, 3 HRIPTs, 25 HMTs and 4 case studies, were identified with cinnamyl alcohol. The positive patch test frequencies from all of the reported patch test studies vary between 0.56 and 100% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0.56 and 1.8% (4 studies) and in studies with selected dermatitis patients positive reactions range between 1.5 and 100% (30 studies). The total number of published cases is > 600. A LOEL (induction) of 4724 µg/cm² was derived from the HRIPT/HMT studies.

Non-human data

A total of 5 LLNAs (two of which were reported to be conducted in accordance with OECD TG 429), 2 GPMT, 1 Freund's complete adjuvant test (FCAT) and 1 Buehler test were identified testing the skin sensitisation of cinnamyl alcohol. EC3 values between 17.9 and 30% were reported for cinnamyl alcohol in the LLNAs and positive reactions were observed in a GPMT (30% positive) and FCAT (15% positive) at intradermal induction doses of 25 and 100%, respectively. No positive reactions were observed in the Buehler test.

No relevant *in vitro* studies on cinnamyl alcohol (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

Exposure

According to data from IFRA (2008a) the exposure of cinnamyl alcohol when used as fragrance in cosmetics and in other consumer products appears to be low.

Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 0.56 and 1.8% with 1/4 studies reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 1.5 and 100% with 28 out of 30 studies reporting frequencies higher than 2%. In addition to this there are more than 600 published cases of positive patch test reactions to cinnamyl alcohol. According to the CLP criteria a frequency $\geq 1\%$ for unselected/consecutive dermatitis patients and/or $\geq 2\%$ for selected dermatitis patients and/or a total number of published cases ≥ 100 , equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that cinnamyl alcohol causes a *high frequency* of occurrence of skin sensitisation based on frequencies in selected dermatitis patients and total number of cases.

In regard to the HRIPT/HMT data the positive response reported at $> 500 \mu\text{g}/\text{cm}^2$ for HRIPT/HMT induction threshold indicate evidence for sub-category 1B according to Annex I: 3.4.2.2.2.2.

In the LLNAs EC3 values between 17.9 and 30% were reported for cinnamyl alcohol. According to the CLP Regulation an EC3 value larger than 2% indicates placement of cinnamyl alcohol into sub-category 1B (Annex I: 3.4.2.2.3.2.).

In the GPMT sensitisation was observed in 30% of the animals after an intradermal induction dose of 25% cinnamyl alcohol. According to the CLP criteria a positive response $\geq 30\%$ of the animals responding at $>1\%$ intradermal induction dose indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.).

No sensitisation was observed in the Buehler test after an induction dose of 30% cinnamyl alcohol.

Overall, there is clear evidence for classification in sub-category 1A based on the frequency of sensitisation in human patch test studies with selected dermatitis patients and the total number of cases combined with the estimated low exposure. Data from HRIPT/HMT indicate evidence for sub-category 1B. All animal studies indicate a classification in sub-category 1B. A classification as a skin sensitizer in sub-category 1A is warranted for cinnamyl alcohol.

Conclusions on classification and labelling

Based on the high frequency of sensitisation observed in human patch test studies and the high number of published cases combined with the estimated low exposure a classification of cinnamyl alcohol as a skin sensitizer in sub-category 1A is justified.

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Appendix 7 Coumarin CAS RN 91-64-5

Non-human information

Table 1 summarises relevant animal studies with coumarin i.e. Local Lymph Node Assays (LLNAs), Guinea Pig Maximization tests (GPMTs) and Buehler tests.

Table 1. Animal studies with coumarin.

Method	Results	Remarks/Comments	Reference
LLNA: 10, 25 and 50% coumarin (purity 99.9%). Vehicle: Dimethyl formamide (DMF).	Coumarin was shown to have a stimulation index (SI) < 3 at all concentrations.	According to SCCS (2012) the study did not deviate from OECD 429 except that coumarin should have been tested in higher concentrations.	Vocanson et al., 2006 cited from SCCS (2012).
LLNA: 5, 10 and 25% Rhodiascent TM Coumarine (purity not reported). Vehicle: 4:1 acetone:olive oil (AOO).	Coumarin was shown to have a stimulation index (SI) < 3 at all concentrations.	According to SCCP (2006) the study was performed in compliance with EEC 96/54/EC Part B, Method B.6. EEC 96/54/EC Part B, Method B.6 is, however, similar to OECD TG 406, which is the GPMT/Buehler test.	CIT 2001 cited from SCCP (2006).
LLNA: 10, 25 and 50% Coumarine Rhodiascent TM (purity not reported). Vehicle: DMF. Three sets of experiments were performed.	Coumarin was shown to have SI < 3 in all experiments except of Experiment 2 at 50% were the SI was 3.1.	According to SCCP (2006) the study was performed in compliance with OECD draft 429 (2000).	INSERM 2003 cited from SCCP (2006).
LLNA: 10, 25 and 50% Coumarine – Chine 0013090/01 Ex PRC (purity not reported). Vehicle: DMF. Three sets of experiments were performed.	Coumarin was shown to have SI < 3 in all experiments except of Experiment 1 at 50% were the SI was 3.	According to SCCP (2006) the study was performed in compliance with OECD draft 429 (2000).	INSERM 2003 cited from SCCP (2006).
LLNA: 10, 25 and 50% Coumarine –	Coumarin was shown to have SI < 3 in all	According to SCCP (2006) the study was performed in	INSERM 2003 cited from SCCP (2006).

Method	Results	Remarks/Comments	Reference
Chine Tianjin freeword (purity not reported). Vehicle: DMF. Three sets of experiments were performed.	experiments except of Experiment 2 at 25 and 50% were the SI was 3.7 and 4, respectively.	compliance with OECD draft 429 (2000).	
LLNA: 1, 2.5, 5 and 10% 6-Chloro- Coumarine (purity not reported). Vehicle: DMF. Three sets of experiments were performed.	Coumarin was shown to have SI < 3 in all experiments except of Experiment 1 at 5 and 10% were the SI was 3.4 and 3.3, respectively.	According to SCCP (2006) the study was performed in compliance with OECD draft 429 (2000).	INSERM 2003 cited from SCCP (2006).
LLNA: 10, 25 and 50% Coumarine Rhodiascent TM (purity not reported). Vehicle: DMF.	Coumarin was shown to have a SI < 3 at all concentrations.	According to SCCP (2006) the study was performed in compliance with OECD draft 429 (2000).	INSERM 2004 cited from SCCP (2006).
LLNA: 10, 25 and 50% Coumarine – Chine 0013090/01 Ex PRC (purity not reported). Vehicle: DMF.	Coumarin was shown to have SI < 3 except at 50% were the SI was 3.19.	According to SCCP (2006) the study was performed in compliance with OECD draft 429 (2000).	INSERM 2004 cited from SCCP (2006).
LLNA: 10, 25 and 50% Coumarine – Chine Tianjin freeword (purity not reported). Vehicle: DMF. Two experiments.	Coumarin was shown to have a SI < 3 at all concentrations.	According to SCCP (2006) the study was performed in compliance with OECD draft 429 (2000).	INSERM 2004 cited from SCCP (2006).
LLNA: 2.5, 5 and 10% 6-Chloro- Coumarine (purity not reported). Vehicle: DMF.	Coumarin was shown to have SI < 3 except at 5% were the SI was 4.94.	According to SCCP (2006) the study was performed in compliance with OECD draft 429 (2000).	INSERM 2004 cited from SCCP (2006).
LLNA: 10, 25 and 50% Coumarine – SRD aromatics LTD –	Coumarin was shown to have a SI < 3 at all concentrations.	According to SCCP (2006) the study was performed in compliance with OECD	INSERM 2004 cited from SCCP (2006).

Method	Results	Remarks/Comments	Reference
Indian no. 2 (purity not reported). Vehicle: DMF.		draft 429 (2000).	
GPMT: Intradermal induction 0.5%; topical induction 25%; challenge dose 25% coumarin. Vehicle not reported.	No positive reactions were observed in the tested animals.	According to REACH-RD (2015h) the study was performed equivalent or similar to OECD TG 406.	Study report from 1979 cited from REACH-RD (2015h).

A total of 20 LLNAs and 1 GPMT with coumarin is summarised in table 1. The majority of LLNAs reported SI values below 3 at concentrations up to 50% coumarin and the SCCS (2012) established an EC3 > 50% for coumarin. Sensitisation was not observed in the GPMT after an intradermal induction dose of 0.5% coumarin.

According to SCCS (2012) “*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.*”

No relevant *in vitro* studies on coumarin (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

Human information

Population studies

Table 2 summarises patch test studies on coumarin involving several thousand dermatitis patients from various countries in Europe. Most of the studies are diagnostic patch test studies.

Table 2. Population studies with coumarin.

Method	Results	Remarks/Comments	Reference
Patch test: Retrospective study of 324 selected patients patch tested with coumarin 5% in petrolatum (pet.).	66/324 (4%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2007- 2009).	Schnuch et al. (2015).
Patch test: Retrospective study of 1951 selected eczema patients patch tested with coumarin 5% in pet.	8/1951 (0.41%, 95% CI: 0.1- 0.7%) patients were positive.	A retrospective study on patch test data at St John’s Institute of Dermatology at St Thomas’ Hospital, UK (2011-2012).	Mann et al. (2014).

Method	Results	Remarks/Comments	Reference
Patch test: Retrospective study of 205 selected patients tested with coumarin 5% in pet.	9/205 (4.4%) patients were positive.	A retrospective study on patch test data from Department of Dermatology, University Hospital St Rafaël, Belgium (1990-2011).	Nardelli et al. (2013).
Patch test: Prospective study of 100 selected patients with contact allergy patch tested with coumarin 5% in pet.	2/100 (2%, 95% CI: 0.24-7.04%) patients were positive.	Single-centre, double-blind prospective experimental longitudinal volunteer study at the department of Dermatology of the VU University Medical Centre, The Netherlands (2005-2010).	Nagtegaal et al. (2012).
Patch test: Prospective study of 565 selected patients patch tested with coumarin 5% in vaseline.	29/565 (5.1%) patients were positive. In addition 8 patients had contact urticaria.	A prospective study on patch test data from multicentre study, Hungary (2009-2010).	Ponyai et al. (2012).
Patch test: Retrospective study of 1503 consecutive eczema patients patch tested with coumarin 5% in pet.	3/1503 (0.7%, 95% CI: 0.1-0.5%) patients were positive.	A retrospective study of patch test data at Department of Dermato-Allergology, Copenhagen University Hospital Gentofte, Denmark (2008-2010).	Heisterberg et al. (2011).
Patch test: Retrospective study of 86 selected patients patch tested with coumarin 5% in pet.	1/86 (1.2%) patients were positive.	A retrospective and descriptive analysis of a patch test study at the Cutaneous Allergy Unit of a tertiary referral hospital, Spain (2004-2008).	Cuesta et al. (2010).
Patch test: Retrospective study of 367 selected FM II positive patients patch tested with coumarin 5% in pet.	10/367 (2.7%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (2005-2008).	Krautheim et al. (2010).
Patch test:	1/18 (6%)	Prospective study of	Pentinga et al.

Method	Results	Remarks/Comments	Reference
Prospective study of 18 selected cinnamon-sensitive patients patch tested with coumarin 5% in pet.	patients were positive.	cinnamon-sensitive patients at the Department of Dermatology of the VU University Medical Centre, The Netherlands (year not stated).	(2009).
Patch test: Prospective study of 320 selected eczema patients patch tested with coumarin 5% in pet.	2/320 (0.6%) patients were positive.	A prospective analysis of selected eczema patients at the University Medical Center in Groningen, the Netherlands (2005-2007).	van Oosten et al. (2009).
Patch test: Retrospective study of 2020 consecutive patients patch tested with coumarin 5%. Vehicle not reported.	8/2020 (0.4%, 95% CI: 0.2-0.8%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (2003-2004).	Schnuch et al. (2007).
Patch test: Study of 252 selected dermatitis patients patch tested with 2% coumarin and 100 selected dermatitis patients patch tested with 1 and 10% coumarin. Vehicle not reported.	1/252 (0.4%) and 0/100 (0%) patients were positive, respectively.	According to the authors no cases of irritancy was observed at 10% coumarin. The number of reported tested subjects is not consistent and the one positive patch test was dismissed by Vocanson 2006 because the patient had "highly sensitive skin".	Vocanson et al. (2006).
Patch test: Study of 101 selected patients allergic to fragrance mix patch tested with coumarin 2%. Vehicle not reported.	1/101 (1%) patients were positive.		Vocanson et al. (2006).
Patch test: Study of 30 selected patients with a positive patch test to their own	0/30 (0%) patients were positive.		Vocanson et al. (2006).

Method	Results	Remarks/Comments	Reference
perfumed product patch tested with coumarin 2%. Vehicle not reported.			
Patch test: Prospective study of 1701 consecutive patients patch tested with coumarin 5% in pet.	0/1701 (0%) patients were positive while 7 doubtful or irritant reactions were observed.	A prospective analysis of patients from six dermatology departments (Dortmund, Copenhagen, Malmö, Odense, London and Leuven) (1997-1998).	Frosch et al. (2005).
Patch test: Prospective study of 1855 consecutive patients patch tested with coumarin 5% in pet.	5/1855 (0.3%) patients were positive.	A prospective analysis of patients from six dermatology departments (Dortmund, Copenhagen, Malmö, Odense, London and Leuven) (1997-1998).	Frosch et al. (2002a).
Patch test: Prospective study of 1825 consecutive patients patch tested with coumarin 5% in pet.	13/1825 (0.7%) patients were positive.	Multicenter study of patch test data in The Netherlands (1998-1999).	de Groot et al. (2000).
Patch test: Study of 14 000 consecutive eczema patients patch tested with coumarin 5% or 8% in pet. (8% for a short period only).	58/14 000 (0.4%) patients were positive.		Kunkeler et al 1998 cited from SCCNFP (1999).
Patch test: Study of 167 selected patients suspected of fragrance sensitivity patch tested with coumarin 5%. Vehicle not reported.	2/167 (1.2%) patients were positive.		Larsen et al 1996 cited from SCCNFP (1999).
Patch test: Study of 119 selected	1/119 (0.8%) patients were		De Groot et al., 1988 cited from

Method	Results	Remarks/Comments	Reference
patients with contact allergy to cosmetic products patch tested with coumarin 5% in pet.	positive.		SCCNFP (1999).
Patch test: Retrospective study of 403 selected patients with cutaneous reactions to cosmetic products patch tested with coumarin. Concentration and vehicle not reported.	4/403 (1%) patients were positive.	It is unclear from the reference exactly how many patients were tested with coumarin.	Adams and Maibach (1985).
Patch test: Study of 242 randomly selected eczema patients patch tested with coumarin 5.8%. Vehicle not reported.	9/242 (3.7%) patients were positive.		Van Joost et al., 1985 cited from SCCNFP (1999).
Patch test: Study of 241 consecutive patients patch tested with coumarin 5%. Vehicle not reported.	2/241 (0.8%) patients were positive.		Ferguson and Shama 1984 cited from SCCNFP (1999).
Patch test: Prospective study of 182 selected patients suspected of contact allergy to cosmetics patch tested with coumarin 8% in pet.	12/182 (6.8%) patients were positive.	Coumarin 8% was tested in 54 controls with no positive reactions.	Malten et al., 1984 cited from SCCNFP (1999).
Patch test: Study of 20 selected perfume allergic patients patch tested with coumarin 5%. Vehicle not reported.	2/20 (10%) patients were positive.		Larsen et al., 1977 cited from SCCNFP (1999).

Case studies

Table 3 summarises case reports with allergic contact dermatitis in different clinics in Europe where coumarin has been found as a causative agent.

Table 3. Case studies with coumarin.

Method	Results	Remarks/Comments	Reference
Patch test: A 44-year old woman with dermatitis after use of deodorant and eau de toilette was tested by ROAT ¹ with chemical fractions of perfume concentrate from her eau de toilette.	Coumarin was confirmed as the allergen by ROAT with 1% after having caused dermatitis by the use of a deodorant containing coumarin at 0.23%.	Case study, Europe (year not stated).	Mutterer et al. (1999).
Patch test: A woman with eczema caused by a perfumed lotion was patch tested with coumarin 0.5% in pet and diluent.	Positive reaction was observed.	Case study, Denmark (year not stated).	Johansen et al., 1994 cited from SCCNFP (1999).

¹ROAT: Repeat Open Application Test

A total of 25 patch test population studies and 2 case studies, one of which included a ROAT, with coumarin are summarised above (Table 2 and 3). As shown in Table 2 the positive patch test frequencies from all of the reported patch test studies vary between 0 and 10% in dermatitis patients. For unselected/consecutive dermatitis patients positive reactions range between 0 and 0.8% (7 studies) and for selected dermatitis patients positive reactions range between 0 and 10% (19 studies). The total number of published cases is > 200.

According to IFRA (2008b) a NOEL¹⁰-HRIPT (induction) of 3543 µg/cm² and a NOEL-HMT (induction) of 5517 µg/cm² has been established for coumarin. On basis of data from HRIPT or HMT (not specified by IFRA (2008b)) a LOEL¹¹ (induction) of 8858 µg/cm² for coumarin was derived.

Coumarin is a "top 100" substance and is according to SCCS (2012) classified as a skin sensitiser with R43 (based on the old classification criteria) (note that the substance does not have a harmonised classification as a skin sensitizer).

According to SCCS (2012) coumarin is used in volumes higher than 175 ton per year in perfume formulations. It has been reported that in consumer products containing fragrance allergens that are required to be labelled 17% of a total of 516 products; 30% of a total of 300 products; ca. 11% of

¹⁰ NOEL: No Observed Effect Level

¹¹ LOEL: Lowest Observed Effect Level

3000 products and 4.8% of children cosmetics were labelled to contain coumarin (Wijnhoven et al., 2008; Buckley, 2007; Schnuch et al., 2009 and Poulsen & Schmidt, 2007 cited from SCCS (2012)). In addition, in 2007, 33% of 88 tested deodorants were labelled to contain coumarin and the fragrance was detected in 52% (range: 3.8-1255 mg/kg) of 23 deodorants selected for analysis (Rastogi et al., 2007 cited from SCCS (2012)).

The IFRA standard limits for coumarin in different IFRA QRA product categories reported by IFRA (2008b and 2015) are shown in table 4.

Table 4. The IFRA standard limits for coumarin in IFRA QRA product categories.

IFRA QRA product category	Product type that drives the category consumer exposure level	IFRA standard limits
Category 1	Lip products	0.1%
Category 2	Deodorants/antiperspirants	0.13%
Category 3	Hydroalcohols for shaved skin	0.5%
Category 4	Hydroalcohols for unshaved skin	1.6%
Category 5	Hand cream	0.8%
Category 6	Mouthwash	2.5%
Category 7	Intimate wipes	0.3%
Category 8	Hair styling aids	2.0% *
Category 9	Rinse-off hair conditioners	5.0% *
Category 10	Hard surface cleaners	2.5% *
Category 11	Candles	Not restricted

IFRA: International Fragrance Association, QRA: Quantitative Risk Assessment.

* Maximum pragmatic level.

Coumarin is registered under the REACH regulation with an annual tonnage band of 1000 - 10 000 tonnes per annum.

Summary and discussion of skin sensitization

Human data

A total of 25 patch test population studies and 2 case studies, one of which included a ROAT, were identified with coumarin. The positive patch test frequencies from all of the reported patch test studies vary between 0 and 10% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0 and 0.8% (7 studies) and in studies with selected dermatitis patients positive reactions range between 0 and 10% (19 studies). The total number of published cases is > 200. A LOEL (induction) of 8858 µg/cm² was derived from the HRIPT/HMT studies.

Non-human data

A total of 20 LLNAs and 1 GPMT were identified testing skin sensitising effects of coumarin. The collected evidence from the LLNAs indicates an EC₃ for coumarin of ca. 50%. Sensitisation was not observed in the GPMT after an intradermal induction dose of 0.5% coumarin.

Contaminants in coumarin may act as weak or moderate sensitizers (SCCS, 2012).

No relevant *in vitro* studies on coumarin (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

Exposure

According to data from IFRA (2008b) the exposure of coumarin when used as fragrance in cosmetics and in other consumer products appears to be low.

Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 0 and 0.8% i.e. all 7 studies reporting frequencies lower than 1%. For selected dermatitis patients positive reactions range between 0 and 10% with 9 out of 19 studies reporting frequencies higher than 2%. In addition to this there are more than 200 published cases of positive patch test reactions to coumarin. According to the CLP criteria a frequency $\geq 1\%$ for unselected/consecutive dermatitis patients and/or $\geq 2\%$ for selected dermatitis patients and/or a total number of published cases ≥ 100 , equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that coumarin causes a *low/moderate frequency* of occurrence of skin sensitisation based on unselected/consecutive dermatitis patients and 10/19 studies with selected dermatitis patients. The remaining studies with selected dermatitis patients (9/19) and number of published cases shows that coumarin causes a *high frequency* of occurrence of skin sensitisation in humans.

In regard to the HRIPT/HMT data the positive response reported at $> 500 \mu\text{g}/\text{cm}^2$ for HRIPT/HMT induction threshold indicate classification of coumarin in sub-category 1B according to Annex I: 3.4.2.2.2.2.

The collected evidence from the LLNAs indicates an EC3 for coumarin of ca. 50%. According to the CLP Regulation an EC3 value $\leq 2\%$ indicates classification of a substance in sub-category 1A whereas an EC3 value $> 2\%$ indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.). Thus, all studies indicate classification of coumarin in sub-category 1B.

The single GPMT with an intradermal induction dose of 0.5% gave no positive reactions which does not justify sub-categorisation (Table 3.4.3).

Overall, there is evidence for classification in sub-category 1A based on the number of cases combined with the estimated low exposure and supported by patch test data from selected dermatitis patients. Data from HRIPT/HMT indicate evidence for sub-category 1B. Data from LLNAs indicate a classification in sub-category 1B. A classification as a skin sensitiser in sub-category 1A is warranted for coumarin.

Conclusions on classification and labelling

Based on the number of cases combined with the estimated low exposure and supported by patch test data from selected dermatitis patients, a classification of coumarin as a skin sensitiser in sub-category 1A is justified.

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Appendix 8 Eugenol CAS RN 97-53-0

According to SCCS (2012) eugenol is a fragrance compound known to be a prohapten and to form sensitising compounds by metabolic transformation.

Non-human information

Table 1 summarises relevant animal studies with eugenol i.e. Local Lymph Node Assays (LLNAs), Guinea Pig Maximization tests (GPMTs) and Buehler tests.

Table 1. Animal studies with eugenol.

Method	Results	Remarks/Comments	Reference
LLNA: BrdU-ELISA 25% eugenol. Vehicle: acetone:olive oil (AOO).	Eugenol was shown to have a mean SI of 2.37	The aim of the study was to validate the use of BALB/c mice in the LLNA:BrdU-ELISA (OECD TG 442B).	Hou et al. (2015).
LLNA: Concentrations of eugenol not specified. Vehicle: AOO.	Eugenol was shown to have an EC3 value of 4.2%.		Strauss et al. (2015).
LLNA: BrdU-ELISA <i>in vivo</i> and <i>ex vivo</i> BrdU. 0, 2, 20 and 40% eugenol. Vehicle: AOO.	Eugenol was shown to have an EC2 value of 8.5% in the BrdU-ELISA <i>in vivo</i> and an EC2 value of 9.5% in the <i>ex vivo</i> BrdU.	Eugenol was classified as positive for skin sensitisation by Williams et al. (2015).	Williams et al. (2015).
LLNA: <i>Ex vivo</i> BrdU. 2.5, 10, 20 and 50% eugenol. Vehicle: AOO.	Eugenol was shown to have an EC3 value of 16.6%.	Eugenol was classified as a weak skin sensitizer by Ulker et al. (2013).	Ulker et al. (2013).
LLNA (OECD TG 429): 2.5, 10 and 25% eugenol. Vehicle: AOO.	Eugenol was shown to have an EC3 value of 4.6%.		Basketter (2012).
LLNA: LLNA:BrdU-FCM. 5, 10 and 25% eugenol. Vehicle: AOO (proportion not specified).	Eugenol was shown to have an EC3 value of 10.1%.	Eugenol was classified as a weak skin sensitizer by Jung (2012).	Jung (2012).
LLNA: 0, 5, 10 and 25% eugenol.	Eugenol was shown to have an EC3 value of		Fukuyama et al. (2010).

Method	Results	Remarks/Comments	Reference
Vehicle: 4:1 AOO.	5.28%.		
LLNA: 1, 3, 10, 30 and 50% eugenol. Vehicle: 3:1 Ethanol:Diethyl phthalate (EtOH:DEP).	Eugenol was shown to have an EC3 value of 5.3% (0.32 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2001f) cited from SCCS (2012).
LLNA: 1, 3, 10, 30 and 50% eugenol. Vehicle: 1:3 EtOH:DEP.	Eugenol was shown to have an EC3 value of 10.5% (0.64 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2001g) cited from SCCS (2012).
LLNA: 1, 3, 10, 30 and 50% eugenol. Vehicle: EtOH.	Eugenol was shown to have an EC3 value of 10.7% (0.65 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2001h) cited from SCCS (2012).
LLNA: 1, 3, 10, 30 and 50% eugenol. Vehicle: DEP.	Eugenol was shown to have an EC3 value of 15.1% (0.92 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2001i) cited from SCCS (2012).
LLNA: 0, 2.5, 5, 10 and 25% eugenol tested in five different laboratories. Vehicle: AOO.	Eugenol was shown to have an EC3 value of 5.8, 14.5, 8.9, 13.8 and 6%, respectively.		Basketter et al. (2007).
LLNA (OECD TG 429): 0, 2.5, 5, 10, 25 and 50% eugenol. Vehicle: 1:3 EtOH:DEP.	Eugenol was shown to have an EC3 value of 5.4%.		Lalko and Api (2006).
LLNA: BrdU-ELISA 0, 1, 6, 15 and 30% eugenol. Vehicle: AOO.	Eugenol was shown to have an EC3 value of 25.1%.		Takeyoshi et al. (2004).
LLNA: 2.5, 5, 10, 25 and 50% eugenol. Vehicle: 4:1 AOO.	Eugenol was shown to have an EC3 value of 11.9% (0.72 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Basketter et al., 1999 cited from SCCS (2012).
GPMT: Intradermal	Positive reactions in 2/10 (20%) of		Takeyoshi et al. (2004).

Method	Results	Remarks/Comments	Reference
induction 5%; topical induction 5%; challenge dose 5% eugenol. Vehicle not reported.	the tested animals.		

A total of 15 LLNAs are summarised in table 1. One study, validating the use of BALB/c mice in the LLNA:BrDU-ELISA (OECD TG 442B), reported a mean stimulation index (SI) of 2.37 (Hou et al., 2015) and one study reported EC2 values of 8.5 and 9.5% (Williams et al., 2015). The remaining 13 studies reported EC3 values between 4.2 and 25.1%. In the single GPMT study a 5% eugenol induction and challenge concentration resulted in positive reactions in 20% of the tested animals.

No relevant *in vitro* studies on eugenol (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

Human information

Population studies

Table 2 summarises patch test studies on eugenol involving several thousand dermatitis patients from various countries in Europe and Asia. Most of the studies are diagnostic patch test studies.

Table 2. Population studies with eugenol.

Method	Results	Remarks/Comments	Reference
Patch test: Retrospective study of 806 selected patients patch tested with eugenol 1% in petrolatum (pet.).	54/806 (6.7%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2007-2009).	Schnuch et al. (2015).
Patch test: Retrospective study of 1951 selected eczema patients patch tested with cinnamaldehyde 2% in pet.	12/1951 (0.62%, 95% CI: 0.3-1%) patients were positive.	A retrospective study on patch test data at St John's Institute of Dermatology at St Thomas' Hospital, UK (2011-2012).	Mann et al. (2014).
Patch test: Retrospective study of 940 selected patients tested with eugenol 1% in pet.	118/940 (12.6%) patients were positive.	A retrospective study on patch test data from Department of Dermatology, University Hospital St Rafaël, Belgium (1990-2011).	Nardelli et al. (2013).
Patch test: Prospective study of 100 selected patients with contact allergy patch tested with eugenol 1% in pet.	7/100 (7%, 95% CI: 2.86-13.89%) patients were positive.	Single-centre, double-blind prospective experimental longitudinal volunteer study at the department of Dermatology of the VU University Medical	Nagtegaal et al. (2012).

Method	Results	Remarks/Comments	Reference
		Centre, The Netherlands (2005-2010).	
Patch test: Retrospective study of 1502 consecutive eczema patients patch tested with eugenol 1% in pet.	4/1502 (0.3%, 95% CI: 0.1-0.6%) patients were positive.	A retrospective study of patch test data at Department of Dermato-Allergology, Copenhagen University Hospital Gentofte, Denmark (2008-2010).	Heisterberg et al. (2011).
Patch test: Retrospective study of 157 selected patients (chosen out of 509 patients positive to fragrance allergens) patch tested with eugenol 5% in pet.	87/157 (55.4%) patients were positive.	A retrospective study of patch test data at the Allergy Clinic of the Department of Dermatology and Venereology, Zagreb University Hospital Center and School of Medicine, Zagreb, Croatia (2001-2005).	Turcic et al. (2011).
Patch test: Retrospective study of 1214 consecutive patients and 4527 selected patients patch tested with eugenol 1%. Vehicle not reported.	5/1214 (0.44%, 95% CI: 0.04-0.84%) and 71/4527 (1.57%, 95% CI: 1.19-1.95%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2005-2008).	Uter et al. (2010).
Patch test: Retrospective study of 86 selected patients patch tested with eugenol 2% in pet.	12/86 (13.9%) patients were positive.	A retrospective and descriptive analysis of a patch test study at the Cutaneous Allergy Unit of a tertiary referral hospital, Spain (2004-2008).	Cuesta et al. (2010).
Patch test: Retrospective study of 167 selected patients patch tested with eugenol 2% in pet.	4/167 (2.4%) patients were positive.	A retrospective study at the Division and School of Allergy and Clinical Immunology, Department of Human Pathology, University of Messina, Italy (year not stated).	Minciullo et al. (2010).
Patch test: Prospective study of 18 selected cinnamon-sensitive patients patch tested with eugenol 2% in pet.	3/18 (17%) patients were positive.	Prospective study of cinnamon-sensitive patients at the Department of Dermatology of the VU University Medical Centre, The Netherlands (year not stated).	Pentinga et al. (2009).

Method	Results	Remarks/Comments	Reference
Patch test: Prospective study of 320 selected eczema patients patch tested with eugenol 2% pet.	4/320 (1.3%) patients were positive.	A prospective analysis of selected eczema patients at the University Medical Center in Groningen, the Netherlands (2005-2007).	van Oosten et al. (2009).
Patch test: Retrospective study of 225 selected patients with a) current allergic dermatitis or b) past allergic dermatitis patch tested with eugenol 1% in pet.	a) 30/225 (13.3%) and b) 53/225 (23.4%) patients were positive.	A retrospective study of patch test data from patients attending the Department of Cutaneous Allergy at St John's Institute of Dermatology, UK (1982-2007).	White (2009).
Patch test: Prospective study of 15 selected patients with eczematous reactions from ketoprofen-containing gels patch tested with eugenol 2%. Vehicle not reported.	2/15 (13.3%) patients were positive.	A prospective study on patch test data from patients from Italy (2006-2007).	Foti et al. (2008).
Patch test: Retrospective study of 2065 unselected patients patch tested with eugenol 1%. Vehicle not reported.	11/2065 (0.5%, 95% CI: 0.2-0.7%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (2003-2004).	Schnuch et al. (2007).
Patch test: Study of 30 selected patients with a positive patch test to their own perfumed product patch tested with eugenol. Concentration and vehicle not reported.	6/30 (20%) patients were positive.		Vocanson et al. (2006).
Patch test: prospective study of 422 selected patients with suspected contact allergy patch tested with eugenol 2% pet.	8/422 (1.9%) patients were positive.	A prospective analysis of patients from nine dermatology departments of university hospitals in Korea (2002-2003).	An et al. (2005).
Patch test: Retrospective study of 4900 unselected patients patch tested with eugenol 1% in pet.	93/4900 (1.9%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (1996-1999).	Schnuch et al. (2002).
Patch test: Retrospective study of 1750 selected patients with suspected	21/1750 (1.2%) patients were positive.	A retrospective analysis of patients from Italy (1998-2000).	Giusti et al. (2001).

Method	Results	Remarks/Comments	Reference
fragrance allergic contact dermatitis patch tested with eugenol 1% in pet.			
Patch test: Prospective study of 747 selected patients with suspected fragrance allergy patch tested with eugenol 1% in pet.	19/747 (2.5%) patients were positive.	A prospective analysis of patients from FAZ-Floridsdorf Allergy Centre, Austria (1997-2000).	Wohrl et al. (2001).
Patch test: Study of 226 selected patients sensitive to fragrance mix (FM) patch tested with eugenol 1% in pet.	33/226 (14.6%) patients were positive.	Department of Dermatology, University Hospital, Coimbra, Portugal (1989-1999)	Brites et al. (2000).
Patch test: Retrospective study of 50 patients sensitive to FM patch tested with eugenol ext. 2% in 1 % sorbitan sesquioleate (SSO).	6/50 (12%) patients were positive.	Retrospective study of patch test data. University Hospital Utrecht, The Netherlands (1994-1998).	Hendriks and van Ginkel (1999).
Patch test: Retrospective study of 40 patients sensitive to FM patch tested with eugenol in pet. Concentration not reported.	2/40 (5%) patients were positive.		Katsarma and Gawkrödger (1999).
Patch test: Study of 167 selected patients suspected of fragrance sensitivity patch tested with eugenol 5%. Vehicle not reported.	13/167 (7.8%) patients were positive.		Larsen et al., 1996 cited from SCCNFP (1999).
Patch test: Prospective study of 1072 consecutive patients patch tested with eugenol 1%. Vehicle not reported.	13/1072 (1.2%) patients were positive.	Prospective study of patients in a multicentre study involving 9 European centres. Year not stated.	Frosch et al., 1995 cited from SCCNFP (1999).
Patch test: Retrospective study of 367 selected patients patch tested with eugenol 1-2% in pet.	30/367 (8.2%) patients were positive.	Retrospective study of patch test data from Department of Dermatology, Gentofte Hospital, Denmark (1979-1983 and 1988-1992).	Johansen and Menne (1995).
Patch test: Prospective study of 50 selected patients positive to a FM	3/50 (6%) patients were positive.	Retrospective study of patch test data from Department of	Becker et al. (1994).

Method	Results	Remarks/Comments	Reference
patch tested with eugenol. Concentration and vehicle not reported.		Dermatology and Venereology, Hungary. Year not stated.	
Patch test: Prospective study of 61 selected patients positive to a FM patch tested with eugenol 5% in pet.	12/61 (19.7%) patients were positive. Control tests in 100 patients not allergic to fragrances showed no positive reactions when tested with eugenol 5% pet.	Prospective study of patch test data from University of Amsterdam and University of Leiden, The Netherlands (1987).	de Groot et al. (1993).
Patch test: Prospective study of 162 selected patients positive to a FM patch tested with eugenol 1% in pet.	11/162 (6.8%) patients were positive.	Prospective study of patch test data from Dermatologische Klinik und Poliklinik, Germany (1991).	Enders et al. (1989).
Patch test: Study of 78 selected patients positive to a FM patch tested with eugenol 2%. Vehicle not reported.	8/78 (10.3%) patients were positive.	Multicentre study involving 6 countries. Year not stated.	Wilkinson et al., 1989 cited from SCCNFP (1999).
Patch test: Study of 156 selected patients with pure contact allergy to cosmetic products patch tested with eugenol. Concentration and vehicle not reported.	11/156 (7.1%) patients were positive.		Broneck et al., 1987 cited from SCCNFP (1999)
Patch test: Retrospective study of 63 selected patients with dermatitis tested positive to perfume mixture patch tested between 1983 and 1984 with eugenol 5% in pet. and 54 selected patients with dermatitis tested positive to perfume mixture patch tested between 1984 and 1985 with eugenol 1% in pet.	Between 1983 and 1984 8/63 (12.7%) and between 1984 and 1985 9/54 (16.7%) patients were positive.	Retrospective study of patch test data from Istituto Dermatologico Santa Maria e San Gallicano, Italy (1983-1985).	Santucci et al. (1987).
Patch test: Retrospective study of 403 selected patients with cutaneous reactions to cosmetic products patch tested with eugenol. Concentration and	4/403 (1%) patients were positive.	It is unclear from the reference exactly how many patients were tested with eugenol.	Adams and Maibach (1985).

Method	Results	Remarks/Comments	Reference
vehicle not reported.			
Patch test: Study of 20 selected perfume allergic patients patch tested with eugenol 2%. Vehicle not reported.	4/20 (20%) patients were positive.		Larsen et al., 1977 cited from SCCNFP (1999).
Patch test and ROAT ¹ : 5 patients tested positive to FM I and eugenol (2%) were tested with a dilution series of eugenol in patch test (17 dilutions) and a ROAT (0.5, 1 and 2.7% eugenol).	4/5 patients were positive to concentrations down to 1.32% eugenol. 4/5 patients became positive to 2.7% eugenol and 1/5 became positive to 1% eugenol in the ROAT.	A prospective analysis of patients from Sweden (year not stated).	Svedman et al. (2012).

¹ROAT: Repeated Open Application Test

Case studies

Table 3 summarises the case reports with allergic contact dermatitis where eugenol has been found as a causative agent.

Table 3. Case studies with eugenol.

Method	Results	Remarks/Comments	Reference
Patch test: A 35-year old dental nurse with vesicular hand eczema and rhinitis was patch tested with eugenol (2% in petrolatum) and an intermediate restorative material (IRM [®] liquid) (10% pet.). The IRM [®] liquid contained >99% eugenol.	Eugenol (2% pet.): weak positive reaction. IRM [®] liquid: ++ patch test reaction.	Case study, Finland (year not stated).	Kanerva et al. (1998).

A total of 36 patch test population studies and 1 case study with eugenol are summarised above (Table 2 and 3). As shown in Table 2 the positive patch test frequencies from all of the reported patch test studies vary between 0.3 and 55.4% in dermatitis patients. For unselected/consecutive dermatitis patients positive reactions range between 0.3 and 1.9% (5 studies) and for selected dermatitis patients positive reactions range between 0.62 and 55.4% (31 studies). The total number of published cases is > 700.

In a study by Svedman and co-workers it was shown that 4/5 patients tested positive to eugenol concentrations down to 1.32% also tested positive to 2.7% eugenol in a Repeated Open Application Test (ROAT) (Svedman et al., 2012).

The Research Institute for Fragrance Materials, Inc. (RIFM) reported a NOEL-HRIPT¹² (induction) of 5906 µg/cm² for eugenol. In addition, based on weight of evidence, a No Expected Sensitization Induction Level (NESIL) of 5900 µg/cm² was established for eugenol by the RIFM Expert Panel (IFRA, 2008c).

According to SCCS (2012) eugenol is used in volumes greater than 175 ton per year in perfume formulations. It has been reported that 15.7% of a total of 516 consumer products; 27% of a total of 300 consumer products, ca. 7.5% of 3000 consumer products and 7.2% of children cosmetics were labelled to contain eugenol (Wijnhoven et al., 2008; Buckley, 2007; Schnuch et al., 2009 and Poulsen and Schmidt, 2007 cited from SCCS (2012)). In addition, in 2007, 27.3% of 88 tested deodorants were labelled to contain eugenol and the fragrance was detected in 30% (range: 1-514 mg/kg) of 23 deodorants selected for analysis (Rastogi et al., 2007 cited from SCCS (2012)). Eugenol is a "top 100" substance and is according to SCCS classified as a skin sensitizer with R43 (based on the old classification criteria) (SCCS, 2012) (note that the substance does not have a harmonised classification as a skin sensitizer).

The IFRA standard limits for eugenol in different IFRA QRA product categories reported by IFRA (2008c and 2015) are shown in table 4.

Table 4. The IFRA standard limits for eugenol in IFRA QRA product categories.

IFRA QRA product category	Product type that drives the category consumer exposure level	IFRA standard limits
Category 1	Lip products	0.2%
Category 2	Deodorants/antiperspirants	0.2%
Category 3	Hydroalcohols for shaved skin	0.5%
Category 4	Hydroalcohols for unshaved skin	0.5%
Category 5	Hand cream	0.5%
Category 6	Mouthwash	4.3%
Category 7	Intimate wipes	0.4%
Category 8	Hair styling aids	0.5%
Category 9	Rinse-off hair conditioners	0.5%
Category 10	Hard surface cleaners	0.5%
Category 11	Candles	Not restricted

IFRA: International Fragrance Association, QRA: Quantitative Risk Assessment.

Eugenol is registered under the REACH regulation with an annual tonnage band of 100 - 1 000 tonnes per annum.

Summary and discussion of skin sensitization

Human data

A total of 36 patch test population studies, 1 ROAT and 1 case study were identified with eugenol. The positive patch test frequencies from all of the reported patch test studies vary between 0.3 and

¹² NOEL-HRIPT: No Observed Effect Level-Human Repeat Insult Patch Test

55.4% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0.3 and 1.9% (5 studies) and in studies with selected dermatitis patients positive reactions range between 0.62 and 55.4% (31 studies). The total number of published cases is > 700. A NESIL from HRIPT studies of 5900 µg/cm² was derived based on weight of evidence by the RIFM Expert Panel.

Non-human data

A total of 15 LLNAs and one GPMT were identified testing the skin sensitisation of eugenol. EC3 values were reported in 13 studies and ranged between 4.2 and 25.1%. Positive reactions (20%) were observed in the GPMT at an intradermal induction dose of 5%.

No relevant *in vitro* studies on eugenol (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

Exposure

According to data from IFRA (2008c) the exposure of eugenol when used as fragrance in cosmetics and in other consumer products appears to be low.

Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 0.3 and 1.9% with 2 out of 5 studies reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 0.62 and 55.4% with 25 out of 31 studies reporting frequencies higher than 2%. In addition to this there are more than 700 published cases of positive patch test reactions to eugenol. According to the CLP criteria a frequency ≥ 1% for unselected/consecutive dermatitis patients and/or ≥ 2% for selected dermatitis patients and/or a total number of published cases ≥ 100, equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that eugenol causes a *high frequency* of occurrence of skin sensitisation based on the frequency of positive reactions mainly in selected dermatitis patients (>2% in 21/27 studies) and the total number of cases.

In regard to the HRIPT/HMT data the positive response reported at > 500 µg/cm² for HRIPT/HMT induction threshold indicate evidence for sub-category 1B according to Annex I: 3.4.2.2.2.2.

In the LLNA tests, EC3 values between 4.2 and 25.1% (13 studies) were reported for eugenol. According to the CLP Regulation an EC3 value larger than 2 indicates placement of eugenol into sub-category 1B (Annex I: 3.4.2.2.3.2.).

In the GPMT sensitisation was observed in 20% of the animals after an intradermal induction dose of 5% which does not justify sub-categorisation into either sub-category 1A or 1B (Annex I: 3.4.2.2.3.2.).

Overall, there is clear evidence for classification in sub-category 1A based on the frequency of sensitisation in human patch test studies mainly with selected dermatitis patients and the total number of cases combined with the estimated low exposure. Data from HRIPT/HMT indicate evidence for sub-category 1B. Data from LLNAs indicate a classification in sub-category 1B. A classification as a skin sensitizer in sub-category 1A is warranted for eugenol.

Conclusions on classification and labelling

Based on the high frequency of sensitisation observed in human patch test studies and the high number of published cases, combined with the estimated low exposure, a classification of eugenol as a skin sensitizer in sub-category 1A is justified.

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Appendix 9 Farnesol CAS RN 4602-84-0

Non-human information

Table 1 summarises relevant animal studies with farnesol i.e. Local Lymph Node Assays (LLNAs), Guinea Pig Maximization tests (GPMTs) and Buehler tests.

Table 1. Animal studies with farnesol.

Method	Results	Remarks/Comments	Reference
LLNA: 5, 10 and 25% farnesol. Vehicle: 4:1 Acetone: Olive Oil (AOO).	Farnesol was shown to have an EC3 value of 5.5% (0.25 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429 except that farnesol should also have been tested at lower concentrations.	Unpublished summary report by RIFM 2009 (RIFM 2004d) cited from SCCS (2012).
LLNA: 5, 10 and 25% farnesol. Vehicle: 4:1 AOO.	Farnesol was shown to have an EC3 value of 4.1% (0.18 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429 except that farnesol should also have been tested at lower concentrations.	Unpublished summary report by RIFM 2009 (RIFM 2004d) cited from SCCS (2012).
GPMT: Intradermal induction 10% farnesol in vaseline diluted in peanut oil; Topical induction 10%; Challenge dose 25, 50 or 100% farnesol in vaseline.	No positive reactions reported.		Unpublished report by RIFM 1983a cited from Lapczynski et al. (2008a).
GPMT: Intradermal induction 5% farnesol in peanut oil; Topical induction 100%; Challenge dose 25% farnesol in peanut oil.	No positive reactions reported.		Unpublished report by RIFM 1995b cited from Lapczynski et al. (2008a).
GPMT: Induction 10%; Challenge dose 10% farnesol.	No positive reactions reported.		Ishihara et al., 1986 cited from Lapczynski et al. (2008a).

Method	Results	Remarks/Comments	Reference
Vehicle not reported.			
GPMT: Induction 0.16%; Challenge dose 0.16% farnesol in acetone.	No positive reactions reported.		Watanbe et al., 1985 cited from Lapczynski et al. (2008a).

A total of two LLNAs and four GPMTs are summarised in table 1. The reported EC3 values in the two LLNAs were 5.5 and 4.1%, respectively. In the GPMTs no positive reactions were observed after intradermal induction doses of 0.16, 5 and 10% farnesol.

No relevant *in vitro* studies on farnesol (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

Human information

Population studies

Table 2 summarises patch test studies on farnesol involving several thousand dermatitis patients from various countries in Europe and Asia. Most of the studies are diagnostic patch test studies.

Table 2. Population studies with farnesol.

Method	Results	Remarks/Comments	Reference
Patch test: Retrospective study of 324 selected patients patch tested with farnesol 5% in petrolatum (pet.).	39/324 (12%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2007-2009).	Schnuch et al. (2015).
Patch test: Retrospective study of 1951 selected eczema patients patch tested with farnesol 5% in pet.	8/1951 (0.41%, 95% CI: 0.1-0.7%) patients were positive.	A retrospective study on patch test data at St John's Institute of Dermatology at St Thomas' Hospital, UK (2011-2012).	Mann et al. (2014).
Patch test: Retrospective study of 205 selected patients tested with farnesol 5% in pet.	27/205 (13.2%) patients were positive.	A retrospective study on patch test data from Department of Dermatology, University Hospital St Rafaël, Belgium (1990-2011).	Nardelli et al. (2013).
Patch test: Prospective study of 100 selected patients with contact allergy patch tested with farnesol 5% in pet.	10/100 (10%, 95% CI: 4.9-17.62%) patients were positive.	Single-centre, double-blind prospective experimental longitudinal volunteer study at the department of Dermatology of the VU	Nagtegaal et al. (2012).

Method	Results	Remarks/Comments	Reference
		University Medical Centre, The Netherlands (2005-2010).	
Patch test: Prospective study of 565 selected patients patch tested with farnesol 5% in vaseline.	14/565 (2.5%) patients were positive.	A prospective study on patch test data from multicentre study, Hungary (2009-2010).	Ponyai et al. (2012).
Patch test: Retrospective study of 1502 consecutive eczema patients patch tested with farnesol 5% in pet.	6/1502 (0.4%) patients were positive.	A retrospective study of patch test data at Department of Dermato-Allergology, Copenhagen University Hospital Gentofte, Denmark (2008-2010).	Heisterberg et al. (2011, 2012).
Patch test: Retrospective study of 86 selected patients patch tested with farnesol 5% in pet.	1/86 (1.2%) patients were positive.	A retrospective and descriptive analysis of a patch test study at the Cutaneous Allergy Unit of a tertiary referral hospital, Spain (2004-2008).	Cuesta et al. (2010).
Patch test: Retrospective study of 367 selected fragrance mix (FM) II positive patients patch tested with farnesol 5% in pet.	42/367 (11.4%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (2005-2008).	Krautheim et al. (2010).
Patch test: Prospective study of 320 selected eczema patients patch tested with farnesol 5% in pet.	3/320 (0.9%) patients were positive.	A prospective analysis of selected eczema patients at the University Medical Center in Groningen, the Netherlands (2005-2007).	van Oosten et al. (2009).
Patch test: Retrospective study of 4238 unselected patients patch tested with farnesol 5% in pet.	38/4238 (0.9%, 95% CI: 0.6-1.2%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (2003-2004).	Schnuch et al. (2007).
Patch test: Prospective study of 1701 consecutive	6/1701 (0.35%, 95% CI: 0.13-0.77%) patients were positive.	A prospective analysis of patients from six dermatology departments	Frosch et al. (2005).

Method	Results	Remarks/Comments	Reference
patients patch tested with farnesol 5% in pet.		(Dortmund, Copenhagen, Malmö, Odense, London and Leuven) (1997-1998).	
Patch test: Prospective study of 1855 consecutive patients patch tested with farnesol 5% in pet.	10/1855 (0.5%) patients were positive.	A prospective analysis of patients from six dermatology departments (Dortmund, Copenhagen, Malmö, Odense, London and Leuven) (1997-1998).	Frosch et al. (2002a).
Patch test: Study of 102 selected patients patch tested with farnesol 5% in pet.	4/102 (4%) patients were positive.		Hausen et al., 2001 cited from Lapczynski et al. (2008a).
Patch test: Study of 1483 selected patients with suspected cosmetic dermatitis patch tested with farnesol 5% in pet.	16/1483 (1.1%) patients were positive.	Nagoya, Japan (year not stated).	Sugiura et al., 2000 cited from SCCS (2012).
Patch test: Retrospective study of 8521 selected patients patch tested with farnesol. Concentration and vehicle not reported.	2/8521 (0.02%) patients were positive.	Retrospective study performed at the Department of Dermatology, University Hospital, Leuven, Belgium (1985-1997).	Goossens and Merckx 1997 cited from SCCNFP (1999).
Patch test: Study of 111 selected patients patch tested with farnesol 1% in lanolin.	8/111 (7.2%) patients were positive.		Goossens and Merckx 1997 cited from SCCNFP (1999).
Patch test: Study of 466 selected patients patch tested with farnesol (2, 5 and 10%). Vehicle not reported.	5/466 (1.1%) patients were positive to 5 or 10% and 1/466 (0.2%) were positive to 2%.	Patch test study performed by the Japanese society of contact dermatitis. Year not stated.	Sugai et al., 1994 cited from SCCNFP (1999).
Patch test: Study of 573 selected patients patch	7/573 (1.2%) patients were positive.		Hirose et al., 1987 cited from Lapczynski et al.

Method	Results	Remarks/Comments	Reference
tested with farnesol 20% in pet.			(2008a).
Patch test: Study of 1367 selected patients patch tested with farnesol 2, 5 or 10% in pet.	11/1367 (0.8%) patients were positive.		Yamamoto et al., 1985 cited from Lapczynski et al. (2008a).
Patch test: Prospective study of 182 selected patients suspected of contact allergy to cosmetics patch tested with farnesol 4% in pet.	2/182 (1.1%) patients were positive.	Farnesol 4% was tested in 20 control eczema patients with no positive reactions.	Malten et al., 1984 cited from SCCNFP (1999).

Table 3 summarises Human Repeat Insult Patch Tests (HRIPTs) and Human Maximisation Tests (HMTs) with farnesol.

Table 3. HRIPT and HMT studies with farnesol adapted from Lapczynski et al. (2008a).

Method	Results	Remarks/Comments	Reference
HRIPT: Farnesol concentration: 5% (2865 µg/cm ²) Vehicle: 3:1 Diethyl phthalate:Ethanol (DEP:EtOH).	0/108 (0%) tests were positive.	No further information available from Lapczynski et al. (2008a).	Unpublished report from RIFM (2004c) cited from Lapczynski et al. (2008a).
HRIPT: Farnesol concentration: 5% (1529 µg/cm ²) Vehicle: petrolatum (pet.).	0/103 (0%) tests were positive.	No further information available from Lapczynski et al. (2008a).	Unpublished report from RIFM (2000b) cited from Lapczynski et al. (2008a).
HRIPT: Farnesol concentration: 5% (1529 µg/cm ²) Vehicle: pet.	0/101 (0%) tests were positive.	No further information available from Lapczynski et al. (2008a).	Unpublished report from RIFM (2000a) cited from Lapczynski et al. (2008a).
HMT: Farnesol concentration: 12% (8280 µg/cm ²) Vehicle: pet.	0/35 (0%) tests were positive.	No further information available from Lapczynski et al. (2008a).	Unpublished report from RIFM (1978) cited from Lapczynski et al. (2008a).

Method	Results	Remarks/Comments	Reference
HMT: Farnesol concentration: 10% (6900 µg/cm ²) Vehicle: pet.	4/25 (16%) tests were positive.	No further information available from Lapczynski et al. (2008a).	Unpublished report from RIFM (1977b) cited from Lapczynski et al. (2008a).
HMT: Farnesol concentration: 12% (8280 µg/cm ²) Vehicle: pet.	0/25 (0%) tests were positive.	No further information available from Lapczynski et al. (2008a).	Unpublished report from RIFM (1977a) cited from Lapczynski et al. (2008a).
HMT: Farnesol concentration: 12% (8280 µg/cm ²) Vehicle: pet.	0/26 (0%) tests were positive.	No further information available from Lapczynski et al. (2008a).	Unpublished report from RIFM (1977a) cited from Lapczynski et al. (2008a).
HMT: Farnesol concentration: 10% (6900 µg/cm ²) Vehicle: pet.	6/25 (24%) tests were positive.	No further information available from Lapczynski et al. (2008a).	Unpublished report from RIFM (1976b) cited from Lapczynski et al. (2008a).
HMT: Farnesol concentration: 10% (6900 µg/cm ²) Vehicle: pet.	0/25 (0%) tests were positive.	No further information available from Lapczynski et al. (2008a).	Unpublished report from RIFM (1976a) cited from Lapczynski et al. (2008a).
HMT: Farnesol concentration: 12% (8280 µg/cm ²) Vehicle: pet.	0/25 (0%) tests were positive.	No further information available from Lapczynski et al. (2008a).	Unpublished report from RIFM (1975d) cited from Lapczynski et al. (2008a).
HMT: Farnesol concentration: 12% (8280 µg/cm ²) Vehicle: pet.	2/25 (8%) tests were positive.	No further information available from Lapczynski et al. (2008a).	Unpublished report from RIFM (1975c) cited from Lapczynski et al. (2008a).
HMT: Farnesol concentration: 12% (8280 µg/cm ²) Vehicle: pet.	0/25 (0%) tests were positive.	No further information available from Lapczynski et al. (2008a).	Unpublished report from RIFM (1975b) cited from Lapczynski et al. (2008a).

Method	Results	Remarks/Comments	Reference
HMT: Farnesol concentration: 12% (8280 µg/cm ²) Vehicle: pet.	7/25 (28%) tests were positive.	No further information available from Lapczynski et al. (2008a).	Unpublished report from RIFM (1975a) cited from Lapczynski et al. (2008a).
HMT: Farnesol concentration: 12% (8280 µg/cm ²) Vehicle: pet.	4/25 (16%) tests were positive.	No further information available from Lapczynski et al. (2008a).	Unpublished report from RIFM (1974a) cited from Lapczynski et al. (2008a).

HRIPT: Human Repeat Insult Patch Test, HMT: Human Maximisation Test.

Case studies

Table 4 summarises case reports with allergic contact dermatitis where farnesol has been found as a causative agent.

Table 4. Case studies with farnesol.

Method	Results	Remarks/Comments	Reference
Patch test: A 48-year old metalworker with recurrent hand dermatitis was patch tested with farnesol and a long list of other allergens.	Farnesol and several other allergens tested positive.	Case study (Germany, 2007-2008).	Tanko et al. (2009).
Patch test: A woman with axillary dermatitis due to a deodorant was patch tested with farnesol.	Positive result at 5% farnesol.	Case study (location and year not stated).	Goossens and Merckx 1997 cited from SCCNFP (1999).

A total of 20 patch test population studies, 3 HRIPTs, 11 HMTs and 2 case studies with farnesol are summarised above (Table 2, 3 and 4). As shown in Table 2 the positive patch test frequencies from all of the reported patch test population studies vary between 0.02 and 13.2% in dermatitis patients. For unselected/consecutive dermatitis patients positive reactions range between 0.35 and 0.9% (4 studies) and for selected dermatitis patients positive reactions range between 0.02 and 13.2% (16 studies). The total number of published cases is > 250. No positive results were reported in the three HRIPTs at farnesol concentrations of 2865 µg/cm² or lower. In the HMTs 2/3 studies with farnesol showed positive results after 6900 µg/cm² and 3/8 studies showed positive results after 8280 µg/cm². Based on these data the Research Institute for Fragrance Materials, Inc. (RIFM) deducted a NOEL¹³-HRIPT (induction) of 2755 µg/cm² and a LOEL¹⁴ (induction) of 68 974

¹³ NOEL: No Observed Effect Level.

¹⁴ LOEL: Lowest Observed Effect Level.

µg/cm². In addition, based on weight of evidence, a No Expected Sensitization Induction Level (NESIL) of 2700 µg/cm² was established for farnesol by the RIFM Expert Panel (IFRA, 2006).

According to SCCS (2012) farnesol is used in volumes less than 175 ton per year in perfume formulations. It has been reported that in consumer products containing fragrance allergens that are required to be labelled 3.9% of a total of 516 consumer products; 8% of a total of 300 consumer products; ca. 4% of 3000 products and 2.9% of children's cosmetics were labelled to contain farnesol (Wijnhoven et al., 2008; Buckley, 2007; Schnuch et al., 2009 and Poulsen & Schmidt, 2007 cited from SCCS (2012)). In addition, in 2007, 14.8% of 88 tested deodorants were labelled to contain farnesol and the fragrance was detected in 39% (range: 9-1791 mg/kg) of 23 deodorants selected for analysis (Rastogi et al., 2007 cited from SCCS (2012)).

The IFRA standard limits for farnesol in different IFRA QRA product categories reported by IFRA (2006 and 2015) are shown in table 5.

Table 5. The IFRA standard limits for farnesol in IFRA QRA product categories.

IFRA QRA product category	Product type that drives the category consumer exposure level	IFRA standard limits
Category 1	Lip products	0.08%
Category 2	Deodorants/antiperspirants	0.11%
Category 3	Hydroalcohols for shaved skin	0.4%
Category 4	Hydroalcohols for unshaved skin	1.2%
Category 5	Hand cream	0.6%
Category 6	Mouthwash	2.0%
Category 7	Intimate wipes	0.2%
Category 8	Hair styling aids	2.0% *
Category 9	Rinse-off hair conditioners	5.0% *
Category 10	Hard surface cleaners	2.5% *
Category 11	Candles	Not restricted

IFRA: International Fragrance Association, QRA: Quantitative Risk Assessment.

*Maximum pragmatic level.

Farnesol is not registered under the REACH regulation.

Summary and discussion of skin sensitization

Human data

A total of 20 patch test population studies, 3 HRIPTs, 11 HMTs and 2 case studies were identified with farnesol. The positive patch test frequencies from all of the reported patch test population studies vary between 0.02 and 13.2% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0.35 and 0.9% (4 studies) and in studies with selected dermatitis patients positive reactions range between 0.02 and 13.2% (16 studies). The total number of published cases is > 250. Positive responses after farnesol were seen at concentrations ≥ 6900 µg/cm² in 5/11 HMTs.

Non-human data

A total of two LLNAs and four GPMTs were identified testing skin sensitising effects of farnesol. EC3 values were 5.5 and 4.1%. In the GPMTs no positive reactions were observed after intradermal induction doses of 0.16, 5 and 10% farnesol.

No relevant *in vitro* studies on farnesol (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

Exposure

According to data from IFRA (2006) the exposure of farnesol when used as fragrance in cosmetics and in other consumer products appears to be low.

Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 0.35 and 0.9% i.e. all 4 studies reporting frequencies lower than 1%. For selected dermatitis patients positive reactions range between 0.02 and 13.2% with 7 out of 16 studies reporting frequencies higher than 2%. In addition to this there are more than 250 published cases of positive patch test reactions to farnesol. According to the CLP criteria a frequency $\geq 1\%$ for unselected/consecutive dermatitis patients and/or $\geq 2\%$ for selected dermatitis patients and/or a total number of published cases ≥ 100 , equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that farnesol causes a *low/moderate frequency* of occurrence of skin sensitisation based on unselected/consecutive dermatitis patients and 9/16 studies with selected dermatitis patients. The remaining studies with selected dermatitis patients (7/16) and number of published cases shows that farnesol causes a *high frequency* of occurrence of skin sensitisation in humans.

In regard to the HRIPT/HMT data the positive response reported at $> 500 \mu\text{g}/\text{cm}^2$ for HRIPT/HMT induction threshold indicate classification of farnesol in sub-category 1B according to Annex I:

3.4.2.2.2.2.

In the two LLNAs the lowest EC3 value for farnesol was 4.1%. According to the CLP Regulation an EC3 value $\leq 2\%$ indicates classification of a substance in sub-category 1A whereas an EC3 value $> 2\%$ indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.). Thus, both studies indicate classification of farnesol in sub-category 1B.

Sensitisation was not observed in the four GPMTs with intradermal induction doses of 0.16, 5 and 10% farnesol which do not justify sub-categorisation (Table 3.4.3).

Overall, there is evidence for classification in sub-category 1A based on the number of cases combined with the estimated low exposure. Data from HRIPT/HMT indicate evidence for sub-category 1B. Data from LLNAs indicate a classification in sub-category 1B. A classification as a skin sensitiser in sub-category 1A is warranted for farnesol.

Conclusions on classification and labelling

Based on the number of cases combined with the estimated low exposure, a classification of farnesol as a skin sensitiser in sub-category 1A is justified.

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Appendix 10 Geraniol CAS RN 106-24-1

Non-human information

Table 1 summarises relevant animal studies with geraniol i.e. Local Lymph Node Assays (LLNAs), Guinea Pig Maximization tests (GPMTs) and Buehler tests.

Table 1. Animal studies with geraniol.

Method	Results	Remarks/Comments	Reference
LLNA: <i>Ex vivo</i> BrdU. 2.5, 10, 20 and 50% geraniol. Vehicle: 4:1 Acetone:Olive oil (AOO).	Geraniol was shown to have an EC3 value of 13.1%.		Ulker et al. (2014).
LLNA: 0, 5, 10, 15, 20 and 30% geraniol. Vehicle: AOO.	Geraniol was shown to have an EC3 value of 22.4% (1.45 M).		Hagvall et al. (2007).
LLNA: 0, 1, 3, 6, 10 and 20% air-exposed geraniol. Vehicle: AOO.	Air-exposed geraniol was shown to have an EC3 value of 4.4% (0.28 M).		Hagvall et al. (2007).
LLNA: 0.5, 1, 3, 6 and 10% air-exposed geraniol. Vehicle: AOO.	Air-exposed geraniol was shown to have an EC3 value of 5.8% (0.37 M).		Hagvall et al. (2007).
LLNA: 1, 3, 10, 30 and 50% geraniol. Vehicle: Ethanol (EtOH).	Geraniol was shown to have an EC3 value of 5.6% (0.36 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2001j) cited from SCCS (2012).
LLNA: 2.5, 5, 10, 25 and 50% geraniol. Vehicle: 3:1 EtOH:diethyl phthalate (DEP).	Geraniol was shown to have an EC3 value of 11.4% (0.74 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2003t) cited from SCCS (2012).
LLNA: 1, 3, 10, 30 and 50% geraniol. Vehicle: DEP.	Geraniol was shown to have an EC3 value of 11.8% (0.76 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2001k) cited from SCCS (2012).

Method	Results	Remarks/Comments	Reference
LLNA: 1, 3, 10, 30 and 50% geraniol. Vehicle: 1:3 EtOH:DEP.	Geraniol was shown to have an EC3 value of 20.4% (1.32 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2001l) cited from SCCS (2012).
LLNA: 1, 3, 10, 30 and 50% geraniol. Vehicle: 3:1 EtOH:DEP.	Geraniol was shown to have an EC3 value of 25.8% (1.67 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2001m) cited from SCCS (2012).
GPMT: Intradermal induction 0.1% geraniol in Dobs/saline; Topical induction 50% in 70/30 acetone/PEG 400; Challenge dose 10% in 70/30 acetone/PEG 400.	No positive reactions reported.		Unpublished report by RIFM 1989 cited from Lapczynski et al. (2008b).
GPMT: Intradermal induction 0.1% geraniol in Dobs/saline; Topical induction 50% in acetone; Challenge dose 10% in acetone.	Sensitization observed.	No further information is available from Lapczynski et al. (2008b).	Unpublished report by RIFM 1989 cited from Lapczynski et al. (2008b).
GPMT: Intradermal induction 5% geraniol in petrolatum (pet.); Topical induction 30% in pet; Challenge dose 10% in pet.	Sensitization observed.	No further information is available from Lapczynski et al. (2008b).	Unpublished report by RIFM 1977 cited from Lapczynski et al. (2008b).
GPMT: Intradermal induction 5% geraniol in pet.; Topical induction 25% in pet; Challenge dose sub-irritant.	Sensitization observed.	No further information is available from Lapczynski et al. (2008b).	Klecak et al., 1977 cited from Lapczynski et al. (2008b).

Method	Results	Remarks/Comments	Reference
GPMT: Intradermal induction 10% geraniol; Topical induction 10%; Challenge dose 10%. Vehicle not reported.	Sensitization observed.	No further information is available from Lapczynski et al. (2008b).	Ishihara et al., 1986 cited from Lapczynski et al. (2008b).
Buehler: Induction concentration 15% geraniol in DEP ; Challenge dose 2.5, 7.5 or 25% in DEP.	No sensitization observed.	No further information is available from Lapczynski et al. (2008b).	Unpublished report by RIFM 1992 cited from Lapczynski et al. (2008b).

A total of 9 LLNAs, 5 GPMTs and 1 Buehler test are summarised in table 1. The reported EC3 values for geraniol range between 5.6% (vehicle: ethanol) and 25.8% (vehicle: ethanol:diethyl phthalate 1:3). Air-exposed geraniol was tested in two LLNAs with resulting EC3 values of 4.4 and 5.8%. In the GPMTs sensitisation was observed but not quantified (i.e. number of animals affected) in 4/5 studies with intradermal induction doses of 0.1, 5 and 10% geraniol. No sensitisation was observed in the Buehler test with an induction concentration of 15% geraniol.

No relevant *in vitro* studies on geraniol (i.e. OECD TG 442C and OECD TG 442D) were identified in the literature.

Human information

Population studies

Table 2 summarises patch test studies on geraniol involving several thousand dermatitis patients from various countries in Europe and Asia. Most of the studies are diagnostic patch test studies.

Table 2. Population studies with geraniol.

Method	Results	Remarks/Comments	Reference
Patch test: Retrospective study of 806 selected patients patch tested with geraniol 1 % in petrolatum (pet.).	31/806 (3.8%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2007- 2009).	Schnuch et al. (2015).
Patch test: Retrospective study of 1951 selected eczema patients patch tested with geraniol 2% in pet.	9/1951 (0.46%, 95% CI: 0.2- 0.8%) patients were positive.	A retrospective study on patch test data at St John's Institute of Dermatology at St Thomas' Hospital, UK (2011-2012).	Mann et al. (2014).

Method	Results	Remarks/Comments	Reference
Patch test: Prospective study of 655 consecutive patients patch tested with geraniol 4 and 11% in pet.	1/655 (0.15%) and 7/655 (1.1%) patients were positive after 4 and 11% geraniol, respectively.	A prospective study on patch test data from Department of Dermatology, Sahlgrenska University Hospital, Gothenburg, Sweden (2010-2011).	Hagvall et al. (2013).
Patch test: Prospective study of 649 consecutive patients patch tested with geraniol 6% in pet.	3/649 (0.46%) patients were positive.	A prospective study on patch test data from Department of Dermatology, Sahlgrenska University Hospital, Gothenburg, Sweden (2010-2011).	Hagvall et al. (2013).
Patch test: Prospective study of 655 consecutive patients patch tested with oxidised geraniol 4 and 6% in pet.	6/655 (0.92%) and 15/655 (2.3%) patients were positive after 4 and 16% geraniol, respectively.	A prospective study on patch test data from Department of Dermatology, Sahlgrenska University Hospital, Gothenburg, Sweden (2010-2011).	Hagvall et al. (2013).
Patch test: Prospective study of 653 consecutive patients patch tested with oxidised geraniol 11% in pet.	30/655 (4.6%) patients were positive.	A prospective study on patch test data from Department of Dermatology, Sahlgrenska University Hospital, Gothenburg, Sweden (2010-2011).	Hagvall et al. (2013).
Patch test: Prospective study of 2227 consecutive patients patch tested with geraniol 2% in pet.	3/2227 (0.13%) patients were positive.	A prospective study on patch test data from Department of Dermatology, Sahlgrenska University Hospital, Gothenburg, Sweden (2006-2010).	Hagvall et al. (2012).
Patch test: Prospective study of 2179 consecutive patients patch tested with oxidised geraniol 2% in pet.	12/2179 (0.55%) patients were positive.	A prospective study on patch test data from Department of Dermatology, Sahlgrenska University Hospital, Gothenburg, Sweden (2006-2010).	Hagvall et al. (2012).
Patch test: Retrospective study of 940 selected patients tested with	52/940 (5.5%) patients were positive.	A retrospective study on patch test data from Department of Dermatology, University Hospital St Rafaël,	Nardelli et al. (2013).

Method	Results	Remarks/Comments	Reference
geraniol 1% in pet.		Belgium (1990-2011).	
Patch test: Prospective study of 100 selected patients with contact allergy patch tested with geraniol. Vehicle and concentration not reported.	9/100 (9%, 95% CI: 4.2-16.4%) patients were positive.	Single-centre, double-blind prospective experimental longitudinal volunteer study at the department of Dermatology of the VU University Medical Centre, The Netherlands (2005-2010).	Nagtegaal et al. (2012).
Patch test: Retrospective study of 1502 consecutive eczema patients patch tested with geraniol 1% in pet.	0/1502 (0 %) patients were positive.	A retrospective study of patch test data at Department of Dermato-Allergology, Copenhagen University Hospital Gentofte, Denmark (2008-2010).	Heisterberg et al. (2011, 2012).
Patch test: Retrospective study of 157 selected patients (chosen out of 509 patients positive to fragrance allergens) patch tested with geraniol 5% in pet.	Ca. 31/157 (ca. 20%) patients were positive.	A retrospective study of patch test data at the Allergy Clinic of the Department of Dermatology and Venereology, Zagreb University Hospital Center and School of Medicine, Zagreb, Croatia (2001-2005).	Turcic et al. (2011).
Patch test: Retrospective study of 86 selected patients patch tested with geraniol 2% in pet.	17/86 (19.7%) patients were positive.	A retrospective and descriptive analysis of a patch test study at the Cutaneous Allergy Unit of a tertiary referral hospital, Spain (2004-2008).	Cuesta et al. (2010).
Patch test: Retrospective study of 1214 consecutive patients and 5695 selected patients patch tested with geraniol in 1% pet.	5/1214 (0.39%, 95% CI: 0.10-0.69%) and 50/5695 (0.87%, 95% CI: 0.63-1.1%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2005-2008).	Uter et al. (2010).
Patch test: Prospective study	2/320 (0.6%) patients were	A prospective analysis of selected eczema	van Oosten et al. (2009).

Method	Results	Remarks/Comments	Reference
of 320 selected eczema patients patch tested with geraniol 2% in pet.	positive.	patients at the University Medical Center in Groningen, the Netherlands (2005-2007).	
Patch test: Retrospective study of 89 selected patients with a) current allergic dermatitis or b) past allergic dermatitis patch tested with geraniol 1% in pet.	a) 15/89 (16.85%) and b) 22/89 (24.72%) patients were positive.	A retrospective study of patch test data from patients attending the Department of Cutaneous Allergy at St John's Institute of Dermatology, UK (1982-2007).	White (2009).
Patch test: Prospective study of 15 selected patients with eczematous reactions from ketoprofen-containing gels patch tested with geraniol 2% in pet.	0/15 (0%) patients were positive.	A prospective study on patch test data from patients from Italy (2006-2007).	Foti et al. (2008).
Patch test: Retrospective study of 2063 unselected patients patch tested with geraniol 1% in pet.	10/2063 (0.5%, 95% CI: 0.1-0.7%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (2003-2004).	Schnuch et al. (2007).
Patch test: Retrospective study of a) 29 patients positive to their own deodorant and b) 141 negative to their own deodorant patch tested with geraniol 1% in pet.	a) 2/29 (7%) and b) 0/141 (0%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (1998-2002).	Uter et al. (2007).
Patch test: Study of 30 selected	6/30 (20%) patients were		Vocanson et al. (2006).

Method	Results	Remarks/Comments	Reference
patients with a positive patch test to their own perfumed product patch tested with geraniol. Concentration and vehicle not reported.	positive.		
Patch test: Study of 658 patients patch tested with geraniol 5% in pet.	6/658 (0.9%) patients were positive.		Heydorn et al., 2003 cited from Lapczynski et al. (2008b).
Patch test: Study of 315 patients patch tested with geraniol 5% in pet.	0/315 (0%) patients were positive.		Heydorn et al., 2002 cited from Lapczynski et al. (2008b).
Patch test: Retrospective study of 4900 unselected patients patch tested with geraniol 1% in pet.	59/4900 (1.2%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (1996-1999).	Schnuch et al. (2002).
Patch test: Study of 160 patients sensitive to fragrance mix (FM) patch tested with geraniol. Concentration and vehicle not reported.	12/160 (7.5%) patients were positive.		Temesvari et al., 2002 cited from Hostynek and Maibach (2004).
Patch test: Prospective study of 747 selected patients with suspected fragrance allergy patch tested with geraniol 1% in pet.	7/747 (0.9%) patients were positive.	A prospective analysis of patients from FAZ-Floridsdorf Allergy Centre, Austria (1997-2000).	Wohrl et al. (2001).
Patch test: Study of 226 patients patch tested with geraniol 1% in	19/226 (8.4%) patients were positive.		Brites et al., 2000 cited from Lapczynski et al. (2008b).

Method	Results	Remarks/Comments	Reference
pet.			
Patch test: Study of 934 patients patch tested with geraniol 1% in pet.	67/934 (7.2%) patients were positive.		Buckley et al., 2000 cited from Lapczynski et al. (2008b).
Patch test: Study of 223 patients patch tested with geraniol 2%. Vehicle not reported.	1/223 (0.4%) patients were positive.		Kiec-Swierczynska & Krecisz 2000 cited from Lapczynski et al. (2008b).
Patch test: Study of 1483 selected patients with suspected cosmetic dermatitis patch tested with geraniol 5% in pet.	5/1483 (0.3%) patients were positive.	Nagoya, Japan (year not stated).	Sugiura et al., 2000 cited from SCCS (2012).
Patch test: Ten-centre study of 542 patients sensitive to FM patch tested with geraniol in pet. Concentration not reported.	58/542 (10.7%) patients were positive.		Bordalo et al., 1999 cited from Hostynek and Maibach (2004).
Patch test: Retrospective study of 50 patients sensitive to FM patch tested with geraniol 2% in 1% sorbitan sesquioleate.	3/50 (6%) patients were positive.	Retrospective study of patch test data. University Hospital Utrecht, The Netherlands (1994-1998).	Hendriks and van Ginkel (1999).
Patch test: Retrospective study of 40 patients sensitive to FM patch tested with geraniol in pet. Concentration not reported.	0/40 (0%) patients were positive.		Katsarma and Gawkrödger (1999).
Patch test: Study of 38 patients sensitive to FM	5/38 (13.2%) patients were positive.		Katsarou 1999 cited from Hostynek and

Method	Results	Remarks/Comments	Reference
patch tested with geraniol 1% in pet.			Maibach (2004).
Patch test: Study of 8 patients sensitive to FM patch tested with geraniol 20% in pet.	3/8 (37.5%) patients were positive.		Goossens & Merckx 1997 cited from Hostynek and Maibach (2004).
Patch test: Study of 41 eczema patients patch tested with geraniol 1% in pet.	1/41 (2.4%) patients were positive.		Schauder & Ippen 1997 cited from Hostynek and Maibach (2004).
Patch test: Study of 167 selected patients suspected of fragrance sensitivity patch tested with geraniol 5%. Vehicle not specified.	5/167 (3%) patients were positive.		Larsen et al., 1996 cited from SCCNFP (1999).
Patch test: Prospective study of 1072 consecutive patients patch tested with geraniol 1% in pet.	4/1072 (0.4%) patients were positive.	Prospective study of patients in a multicentre study involving 9 European centres. Year not stated.	Frosch et al., 1995a cited from SCCNFP (1999).
Patch test: Multicentre study of 702 eczema patients patch tested with geraniol 1% in pet.	5/702 (0.7%) with SSO and 3/702 (0.4%) without SSO patients were positive.	No further information available from Hostynek and Maibach (2004).	Frosch et al., 1995b cited from Hostynek and Maibach (2004).
Patch test: Retrospective study of 367 selected patients patch tested with geraniol in 1-2% pet.	15/367 (4%) patients were positive.	Retrospective study of patch test data from Department of Dermatology, Gentofte Hospital, Denmark (1979-1983 and 1988-1992).	Johansen and Menne (1995).
Patch test: Prospective study	3/50 (6%) patients were	Retrospective study of patch test data from	Becker et al. (1994).

Method	Results	Remarks/Comments	Reference
of 50 selected patients sensitive to FM patch tested with geraniol. Concentration and vehicle not reported.	positive.	Department of Dermatology and Venereology, Hungary. Year not stated.	
Patch test: Prospective study of 61 selected patients sensitive to FM patch tested with geraniol 5% in pet.	8/61 (13%) patients were positive. Control tests in 100 patients not allergic to fragrances showed no positive reactions when tested with geraniol 5% pet.	Prospective study of patch test data from University of Amsterdam and University of Leiden, The Netherlands (1987).	de Groot et al. (1993).
Patch test: Study of 103 patients patch tested with geraniol 5% in pet.	4/103 (3.9%) patients were positive.		Haba et al., 1993 cited from Lapczynski et al. (2008b).
Patch test: Study of 20 volunteers (age: 1-18 years) patch tested with geraniol 1% in pet.	0/20 (0%) patients were positive.	The study by Adifadel et al 1992 has a reliability score of 2 according to REACH-RD(2015i).	Abifadel et al., 1992 cited from REACH-RD (2015i).
Patch test: Study of 111 patients patch tested with geraniol 5% pet.	1/111 (0.9%) patients were positive.		Nagareda et al., 1992 cited from Lapczynski et al. (2008b).
Patch test: Study of 115 patients patch tested with geraniol 5% in vaseline.	0/115 (0%) patients were positive.		Remaut 1992 cited from Lapczynski et al. (2008b).
Patch test: Multi-centre study of 17 patients sensitive to FM patch tested with geraniol 1% in pet.	2/17 (12%) patients were positive.		Roesyanto-Mahadi et al., 1990 cited from Hostynek and Maibach (2004).
Patch test: Study of 20 patients sensitive to fragrance patch	2/20 (10%) patients were positive.		Safford et al., 1990 cited from Hostynek and Maibach (2004).

Method	Results	Remarks/Comments	Reference
tested with geraniol 2% in pet.			
Patch test: Prospective study of 162 selected patients sensitive to FM patch tested with geraniol 1%. Vehicle not reported.	4/162 (2.5%) patients were positive.	Retrospective study of patch test data from Dermatologische Klinik und Poliklinik, Germany (1987).	Enders et al. (1989).
Patch test: Study of 200 patients sensitive to FM patch tested with geraniol 1-3% in pet.	14/200 (7%) patients were positive.		Malanin & Ohela 1989 cited from Hostynek and Maibach (2004).
Patch test: Study of 52 eczema patients patch tested with geraniol 2% in pet.	2/52 (3.8%) patients were positive.		Nethercott et al., 1989 cited from Hostynek and Maibach (2004).
Patch test: Study of 78 selected patients sensitive to FM patch tested with geraniol 1%. Vehicle not reported.	4/78 (5.1%) patients were positive.	Multicentre study involving 6 countries. Year not stated.	Wilkinson et al., 1989 cited from SCCNFP (1999).
Patch test: Study of 119 patients patch tested with geraniol 5% in pet.	2/119 (1.7%) patients were positive.		De Groot et al., 1988 cited from Lapczynski et al. (2008b).
Patch test: Study of 31 patients sensitive to oak moss patch tested with geraniol. Vehicle not reported.	5/31 (16%) patients were positive.		Goncalo et al., 1988 cited from Hostynek and Maibach (2004).
Patch test: Study of 156 selected patients with pure contact allergy to cosmetic products patch tested with	2/156 (1.2%) patients were positive.		Broneck et al., 1987 cited from SCCNFP (1999).

Method	Results	Remarks/Comments	Reference
geraniol. Concentration and vehicle not reported.			
Patch test: Study of 574 selected patients patch tested with geraniol 20% in pet.	5/574 (0.9%) patients were positive.		Hirose et al., 1987 cited from Lapczynski et al. (2008b).
Patch test: Retrospective study of 63 selected patients with dermatitis tested positive to perfume mixture patch tested between 1983 and 1984 with geraniol 3% in pet. and 54 selected patients with dermatitis tested positive to perfume mixture patch tested between 1984 and 1985 with geraniol 1% in pet.	Between 1983 and 1984 4/63 (6.3%) and between 1984 and 1985 4/54 (7.4%) patients were positive.	Retrospective study of patch test data from Istituto Dermatologico Santa Maria e San Gallicano, Italy (1983-1985).	Santucci et al. (1987).
Patch test: Study of 830 patients patch tested with geraniol 5%. Vehicle not reported.	6/830 (0.7%) patients were positive.		Itoh et al., 1986 cited from Lapczynski et al. (2008b).
Patch test: Study of 299 patients sensitive to FM patch tested with geraniol 2% in pet.	10/299 (3.3%) patients were positive.		Rudzki & Grzywa 1986 cited from Hostynek and Maibach (2004).
Patch test: Retrospective study of 403 selected patients with cutaneous reactions to cosmetic products patch tested with	8/403 (2%) patients were positive.	It is unclear from the reference exactly how many patients were tested with geraniol.	Adams and Maibach (1985).

Method	Results	Remarks/Comments	Reference
geraniol. Concentration and vehicle not reported.			
Patch test: Study of 144 patients sensitive to FM patch tested with geraniol 1% in pet.	10/144 (7%) patients were positive.		Angelini et al., 1985 cited from Hostynek and Maibach (2004).
Patch test: Study of 1033 patients patch tested with geraniol 2% pet.	6/1033 (0.6%) patients were positive.		Cronin 1985 cited from Lapczynski et al. (2008b).
Patch test: Study of 179 patients patch tested with geraniol 10% in pet.	11/179 (6.2%) patients were positive.		De Groot et al., 1985 cited from Lapczynski et al. (2008b).
Patch test: Study of 50 cosmetic allergic patients patch tested with geraniol 5% in pet.	20/50 (40%) patients were positive.		Emmons and Marks 1985 cited from Hostynek and Maibach (2004).
Patch test: Study of 242 patients patch tested with geraniol 7%. Vehicle not reported.	1/242 (0.4%) patients were positive.		Van Joost et al., 1985 cited from Lapczynski et al. (2008b).
Patch test: Study of 241 patients patch tested with geraniol 2% in yellow paraffin.	10/241 (4.2%) patients were positive.		Ferguson and Sharma 1984 cited from SCCNFP (1999).
Patch test: Prospective study of 182 selected patients suspected of contact allergy to cosmetics patch tested with geraniol 1% in pet.	3/182 (1.6%) patients were positive.		Malten et al., 1984 cited from SCCNFP (1999).
Patch test: Study of 522 patients patch tested with	3/522 (0.6%) patients were positive.		Nishimura et al., 1984 cited from Lapczynski et al.

Method	Results	Remarks/Comments	Reference
geraniol 5%. Vehicle not reported.			(2008b).
Patch test: Study of 242 patients patch tested with geraniol 1%. Vehicle not reported.	1/242 (0.4%) patients were positive.		Van Joost et al., 1984 cited from Lapczynski et al. (2008b).
Patch test: Study of 181 patients patch tested with geraniol 20% in pet.	7/181 (3.9%) patients were positive.		Hayakawa et al., 1983 cited from Lapczynski et al. (2008b).
Patch test: Study of 467 patients patch tested with geraniol 2%. Vehicle not reported.	1/467 (0.2%) patients were positive.		Ohela and Saramies 1983 cited from Lapczynski et al. (2008b).
Patch test: Study of 23 fragrance sensitive patients patch tested with geraniol 1% in pet.	3/23 (13%) patients were positive.		Sugai 1983 cited from Hostynek and Maibach (2004).
Patch test: Study of 539 patients patch tested with geraniol 2% yellow paraffin.	8/539 (1.5%) patients were positive.		Addo et al., 1982 cited from Lapczynski et al. (2008b).
Patch test: Prospective multicentre study of 487 patients allergic to cosmetics patch tested with geraniol. Concentration and vehicle not reported.	5/487 (1%) patients were positive.		Eiermann et al., 1982 cited from Hostynek and Maibach (2004).
Patch test: Study of 155 patients patch tested with geraniol 5% in pet.	1/155 (0.6%) patients were positive.		Itoh 1982 cited from Lapczynski et al. (2008b).
Patch test: Study	28/1277 (2.2%)		Sugai 1982 cited

Method	Results	Remarks/Comments	Reference
of 1277 patients patch tested with geraniol 2% in pet.	patients were positive.		from Lapczynski et al. (2008b).
Patch test: Study of 172 patients patch tested with geraniol 2% pet.	7/172 (4%) patients were positive.		Calnan et al., 1980 cited from Lapczynski et al. (2008b).
Patch test: Study of 198 patients patch tested with geraniol 2% vaseline.	0/198 (0%) patients were positive.		Ishihara et al., 1979 cited from Lapczynski et al. (2008b).
Patch test: Study of 198 patients patch tested with geraniol 5% in vaseline.	3/198 (1.5%) patients were positive.		Ishihara et al., 1979 cited from Lapczynski et al. (2008b).
Patch test: Study of 20 selected perfume allergic patients patch tested with geraniol 5%. Vehicle not reported.	6/20 (30%) patients were positive.		Larsen et al., 1977 cited from SCCNFP (1999).
Patch test: Study of 792 eczema patients patch tested with geraniol 10% in pet.	4/792 (0.5%) patients were positive.		Fregert and Hjorth 1969 cited from Hostynek and Maibach (2004).
Patch test: Study of 15 eczema patients allergic to Balsam of Peru patch tested with geraniol 10% in pet.	2/15 (13%) patients were positive.		Hjorth 1961 cited from Hostynek and Maibach (2004).
Patch test: Study of 3 eczema patients patch tested with geraniol 1% in acetone.	1/3 (33%) patients were positive.		Keil 1947 cited from Hostynek and Maibach (2004).

Table 3 summarises Human Repeat Insult Patch Tests (HRIPTs) and Human Maximisation Tests (HMTs) with geraniol.

Table 3. HRIPT and HMT studies with geraniol adapted from Lapczynski et al. (2008b).

Method	Results	Remarks/Comments	Reference
HRIPT: Geraniol concentration: 2% (2362 µg/cm ²) Vehicle: 3:1 Diethyl phthalate:Ethanol (DEP:EtOH).	0/110 (0%) tests were positive.	No further information available from Lapczynski et al. (2008b).	Unpublished report from RIFM (2000) cited from Lapczynski et al. (2008b).
HRIPT: Geraniol concentration: 5% (5905 µg/cm ²) plus 0.5% tocopherol Vehicle: 3:1 DEP:EtOH.	1/109 (0.9%) tests were positive.	No further information available from Lapczynski et al. (2008b).	Unpublished report from RIFM (2002) cited from Lapczynski et al. (2008b).
HRIPT: Geraniol concentration: 10% (11810 µg/cm ²) Vehicle: 3:1 DEP:EtOH.	3/112 (2.7%) tests were positive.	No further information available from Lapczynski et al. (2008b).	Unpublished report from RIFM (2004) cited from Lapczynski et al. (2008b).
HRIPT: Geraniol concentration: 5% (3876 µg/cm ²) Vehicle: alcohol SDA 39C.	0/40 (0%) tests were positive.	No further information available from Lapczynski et al. (2008b).	Unpublished report from RIFM (1964) cited from Lapczynski et al. (2008b).
HRIPT: Geraniol concentration: 12.5% (9690 µg/cm ²) Vehicle: EtOH.	0/41 (0%) tests were positive.	No further information available from Lapczynski et al. (2008b).	Unpublished report from RIFM (1964a) cited from Lapczynski et al. (2008b).
HRIPT (modified): Geraniol concentration: 10% Vehicle: petrolatum (pet.).	0/104 (0%) tests were positive.	No further information available from Lapczynski et al. (2008b).	Marzulli and Maibach 1980 cited from Lapczynski et al. (2008b).
HRIPT (modified): Geraniol concentration: 10% Vehicle: alcohol.	2/73 (2.7%) tests were positive.	No further information available from Lapczynski et al. (2008b).	Marzulli and Maibach 1980 cited from Lapczynski et al.

Method	Results	Remarks/Comments	Reference
			(2008b).
HMT: Geraniol concentration: 6% Vehicle: not reported.	0/25 (0%) tests were positive.	No further information available from REACH-RD (2015j). This study was administered a reliability score of 4 (not assignable) in REACH-RD (2015j).	Study report from 1986 cited from REACH-RD (2015j).
HMT: Geraniol concentration: 6% (4140 µg/cm ²) Vehicle: pet.	0/24 (0%) tests were positive.	No further information available from Lapczynski et al. (2008b).	Unpublished report from RIFM (1979) cited from Lapczynski et al. (2008b).
HMT: Geraniol concentration: 6% (4140 µg/cm ²) Vehicle: pet.	0/25 (0%) tests were positive.	No further information available from Lapczynski et al. (2008b).	Grief (1967) cited from Lapczynski et al. (2008b).
HMT: Geraniol concentration: 6% (4140 µg/cm ²) Vehicle: pet.	1/26 (3.8%) tests were positive.	No further information available from Lapczynski et al. (2008b).	Unpublished report from RIFM (1979a) cited from Lapczynski et al. (2008b).

HR IPT: Human Repeat Insult Patch Test, HMT: Human Maximisation Test.

Case studies

Table 4 summarises case reports with allergic contact dermatitis where geraniol has been found to be among the causative agents.

Table 4. Case studies with geraniol.

Method	Results	Remarks/Comments	Reference
Patch test: A 54-year old female bartender with chronic hand dermatitis was patch tested with geraniol and a long list of other allergens present in her environment.	Geraniol, lime peel, FM I and FM II tested positive.	Case study (year not reported).	Swerdlin et al. (2010).
Patch test: A 48-year old male metalworker with	Geraniol and several other allergens tested	Case study (Germany, 2007-2008).	Tanko et al. (2009).

recurrent hand dermatitis was patch tested with geraniol and a long list of other allergens. positive.

A total of 84 results from patch test population studies, 7 HRIPTs, 4 HMTs and 2 case studies with geraniol are summarised above (Table 2, 3 and 4). In addition there were four patch test studies with oxidised geraniol. As shown in Table 2 the positive patch test frequencies from all of the reported patch test population studies vary between 0 and 40% in dermatitis patients. For unselected/consecutive dermatitis patients, positive reactions range between 0 and 1.2% (10 studies) and for selected dermatitis patients positive reactions range between 0 and 40% (74 studies). The total number of published cases is > 900.

Sensitisation (2.7%) was reported in a HRIPT at a geraniol concentration of 11 810 µg/cm² (10%) and in a modified HRIPT at 10% geraniol. Geraniol (5905 µg/cm²) and tocopherol (0.5%) lead to sensitisation in 1/109 subjects. In the HMTs 1/4 studies with geraniol showed a positive result after 4140 µg/cm². Based on these data the Research Institute for Fragrance Materials, Inc. (RIFM) deducted a NOEL-HRIPT¹⁵ (induction) of 11 811 µg/cm². In addition, based on weight of evidence, a No Expected Sensitization Induction Level (NESIL) of 11 800 µg/cm² was established for geraniol by the RIFM Expert Panel (IFRA, 2007).

Geraniol is a "top 100" substance and is according to SCCS (2012) classified as a skin sensitizer with R43 (based on the old classification criteria) (note that the substance does not have a harmonised classification as a skin sensitizer).

Geraniol is identified as a prehapten (compounds which sensitization potency are markedly increased by air exposure due to oxidation) and forms oxidation products with increased sensitizing capacity both via spontaneous autooxidation at air exposure and via metabolic oxidation (SCCS, 2012).

According to SCCS (2012) geraniol is used in volumes greater than 175 ton per year in perfume formulations. It has been reported that in consumer products containing fragrance allergens that are required to be labelled 22.1% of a total of 516 consumer products; 42% of a total of 300 consumer products; ca. 20% of 3000 products and 12% of children's cosmetics were labelled to contain geraniol (Wijnhoven et al., 2008; Buckley, 2007; Schnuch et al., 2009 and Poulsen & Schmidt, 2007 cited from SCCS (2012)). In addition, in 2007, 48.9% of 88 tested deodorants were labelled to contain geraniol and the fragrance was detected in 87% (range: 1-399 mg/kg) of 23 deodorants selected for analysis (Rastogi et al., 2007 cited from SCCS (2012)).

In a recent study the Acceptable Exposure Level (AEL) was estimated for geraniol based on human studies and LLNA data. Comparing the AEL with the estimated aggregate dermal exposure of geraniol from personal care products and household cleaning agents it was shown that between 0.02 and 0.86% of the population may have an aggregated exposure of geraniol which exceeds the lowest AEL of 55 µg/cm² (Nijkamp et al., 2015).

The IFRA standard limits for geraniol in different IFRA QRA product categories reported by IFRA (2007 and 2015) are shown in table 5.

¹⁵ NOEL-HRIPT: No Observed Effect Level-Human Repeat Insult Patch Test.

Table 5. The IFRA standard limits for geraniol in IFRA QRA product categories.

IFRA QRA product category	Product type that drives the category consumer exposure level	IFRA standard limits
Category 1	Lip products	0.3%
Category 2	Deodorants/antiperspirants	0.4%
Category 3	Hydroalcohols for shaved skin	1.8%
Category 4	Hydroalcohols for unshaved skin	5.3%
Category 5	Hand cream	2.8%
Category 6	Mouthwash	8.6%
Category 7	Intimate wipes	0.9%
Category 8	Hair styling aids	2.0% *
Category 9	Rinse-off hair conditioners	5.0% *
Category 10	Hard surface cleaners	2.5% *
Category 11	Candles	Not restricted

IFRA: International Fragrance Association, QRA: Quantitative Risk Assessment.

*Maximum pragmatic level.

Geraniol is registered under the REACH regulation with an annual tonnage band of 1000 - 10 000 tonnes per annum.

Summary and discussion of skin sensitization

Human data

A total of 84 results from patch test population studies, 7 HRIPTs, 4 HMTs and 2 case studies were identified with geraniol. The positive patch test frequencies from all of the reported patch test population studies vary between 0 and 40% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0 and 1.2% (10 studies) and in studies with selected dermatitis patients positive reactions range between 0 and 40% (74 studies). The total number of published cases is > 900. Sensitisation was reported in 2/4 HRIPT studies after exposure to 10% geraniol (11 810 µg/cm²) and in 1/4 HMT studies after exposure to 4140 µg/cm².

Non-human data

A total of 9 LLNAs, 5 GPMTs and 1 Buehler test were identified testing skin sensitising effects of geraniol. The reported EC3 values for geraniol ranged between 5.6% and 25.8% in different vehicles. In the GPMTs sensitisation was observed but not quantified (i.e. number of animals affected) in 4/5 studies with intradermal induction doses of 0.1, 5 and 10% geraniol. No sensitisation was observed in 1/5 GPMTs with an induction concentration of 50% geraniol and in the Buehler test with an induction concentration of 15% geraniol. No relevant *in vitro* studies on geraniol (i.e. OECD TG 442C and OECD TG 442D) were identified in the literature.

Exposure

According to data from IFRA (2007) the exposure of geraniol when used as fragrance in cosmetics and in other consumer products appears to be relatively low. A recent study has indicated that up to 0.86% of the population might be exposed to geraniol from personal care products and household cleaning agents at levels exceeding the estimated Acceptable Exposure Level of 55 µg/cm² (Nijkamp et al., 2015).

Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 0 and 1.2% with 2/10 studies reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 0 and 40% with 44 out of 74 studies reporting frequencies higher than 2%. In addition to this there are more than 900 published cases of positive patch test reactions to geraniol. According to the CLP criteria a frequency $\geq 1\%$ for unselected/consecutive dermatitis patients and/or $\geq 2\%$ for selected dermatitis patients and/or a total number of published cases ≥ 100 , equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that geraniol causes a *high frequency* of occurrence of skin sensitisation based on patch test data mainly from selected dermatitis patients and the number of published cases.

In regard to the HRIPT/HMT data the positive response reported at $> 500 \mu\text{g}/\text{cm}^2$ for HRIPT/HMT induction threshold indicate classification of geraniol in sub-category 1B according to Annex I:

3.4.2.2.2.2.

In the LLNAs EC3 values between 5.6 (vehicle: ethanol) and 25.8% (vehicle: ethanol:diethyl phthalate 1:3) were reported for geraniol. According to the CLP Regulation an EC3 value larger than 2% indicates classification of geraniol in sub-category 1B.

In the GPMTs sensitisation was reported to be observed but not quantified (i.e. number of animals affected) in 4/5 studies with intradermal induction doses of 0.1, 5 and 10% geraniol, therefore, these GPMTs cannot be compared with the classification criteria.

No sensitisation was observed in the Buehler test with an induction concentration of 15% geraniol.

Overall, there is clear evidence for classification in sub-category 1A based on the frequency of sensitisation in human patch test studies mainly with selected dermatitis patients and the total number of cases combined with the estimated relatively low exposure. Data from HRIPT/HMT indicate evidence for sub-category 1B. LLNAs indicate a classification in sub-category 1B. A classification as a skin sensitizer in sub-category 1A is warranted for geraniol.

Conclusions on classification and labelling

Based on the high frequency of sensitisation observed in human patch test studies and the high number of published cases, combined with the estimated relatively low exposure, a classification of geraniol as a skin sensitizer in sub-category 1A is justified.

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Appendix 11 7-Hydroxycitronellal CAS RN 107-75-5

Non-human information

Table 1 summarises relevant animal studies with 7-hydroxycitronellal i.e. Local Lymph Node Assays (LLNAs), Guinea Pig Maximization tests (GPMTs) and Buehler tests.

Table 1. Animal studies with 7-hydroxycitronellal.

Method	Results	Remarks/Comments	Reference
LLNA: <i>Ex vivo</i> BrdU. 0.5, 1, 5 and 10% 7-hydroxycitronellal. Vehicle: 4:1 Acetone:Olive oil (AOO).	7-hydroxycitronellal was shown to have an EC3 value of 9.8%.		Ulker et al. (2014).
LLNA: 1, 3, 10, 30 and 50% 7-hydroxycitronellal. Vehicle: 1:3 ethanol:diethyl phthalate (EtOH:DEP).	7-hydroxycitronellal was shown to have an EC3 value of 19.3% (1.12 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2001n) cited from SCCS (2012).
LLNA: 1, 3, 10, 30 and 50% 7-hydroxycitronellal. Vehicle: DEP.	7-hydroxycitronellal was shown to have an EC3 value of 19.7% (1.14 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2001o) cited from SCCS (2012).
LLNA: 1, 3, 10, 30 and 50% 7-hydroxycitronellal. Vehicle: 3:1 EtOH:DEP.	7-hydroxycitronellal was shown to have an EC3 value of 22.2% (1.29 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2001p) cited from SCCS (2012).
LLNA: 1, 3, 10, 30 and 50% 7-hydroxycitronellal. Vehicle: EtOH.	7-hydroxycitronellal was shown to have an EC3 value of 26.4% (1.53 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2001q) cited from SCCS (2012).
LLNA: 2.5, 5, 10, 25 and 50% 7-hydroxycitronellal. Vehicle: 4:1 AOO.	7-hydroxycitronellal was shown to have an EC3 value of 33% (1.92 M).	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Basketter et al., 2001 cited from SCCS (2012).
LLNA: 10 and 25% 7-	7-	7-hydroxycitronellal	Smith and

Method	Results	Remarks/Comments	Reference
hydroxycitronellal. Vehicle: 4:1 AOO.	hydroxycitronellal was shown to have an EC3 value of 23% (1.34 M).	was only tested in two concentrations.	Hotchkiss 2001 cited from SCCS (2012).
GPMT: Intradermal induction 0.5% 7-hydroxycitronellal; Topical induction 100% 7-hydroxycitronellal; Challenge dose 50% 7-hydroxycitronellal. Vehicle: 70:30 acetone:polyethylene glycol 400.	60% of the animals were positive (total number of animals not reported).	According to REACH-RD (2015a) the study is reliable with restrictions (reliability 2) and performed according to Magnusson and Kligman (1970).	Basketter and Scholes 1992 cited from REACH-RD (2015a).
Buehler: Induction concentration 10 and 30% 7-hydroxycitronellal; Challenge dose 3 and 10% 7-hydroxycitronellal. Vehicle: EtOH (induction), acetone (challenge).	30% induction: 3/8 (38%) animals showed sensitising effects. 10% induction: 0/8 (0%) animals showed sensitising effects.	According to REACH-RD (2015b) the study is reliable with restrictions (reliability 2) and performed according to Buehler (1965).	Buehler 1985 cited from REACH-RD (2015b).

A total of 7 LLNAs including 1 LLNA *ex vivo* BrdU, 1 GPMT and 1 Buehler test with 7-hydroxycitronellal are summarised in table 1. The reported EC3 values for 7-hydroxycitronellal range between 9.8% and 33% both with acetone:olive oil (4:1) as vehicle. In the GPMT sensitisation in 60% of the animals (number of animals not reported) after an intradermal induction dose of 0.5% 7-hydroxycitronellal. Sensitisation was also observed in 38% of the animals in the Buehler test with an induction concentration of 30% 7-hydroxycitronellal. No sensitisation was observed after 10% 7-hydroxycitronellal in the Buehler test.

No relevant *in vitro* studies on 7-hydroxycitronellal (i.e. OECD TG 442C and OECD TG 442D) were identified in the literature.

Human information

Population studies

Table 2 summarises patch test studies on 7-hydroxycitronellal involving several thousand dermatitis patients from various countries in Europe and Asia. Most of the studies are diagnostic patch test studies.

Table 2. Population studies with 7-hydroxycitronellal.

Method	Results	Remarks/Comments	Reference
Patch test: Retrospective study of 806 selected patients patch tested with 7-hydroxycitronellal 1% in petrolatum (pet.).	77/806 (9.6%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2007-2009).	Schnuch et al. (2015).
Patch test: Retrospective study of 1951 selected eczema patients patch tested with 7-hydroxycitronellal in 2% pet.	20/1951 (1%, 95% CI: 0.6-1.4%) patients were positive.	A retrospective study on patch test data at St John's Institute of Dermatology at St Thomas' Hospital, UK (2011-2012).	Mann et al. (2014).
Patch test: Retrospective study of 940 selected patients tested with 7-hydroxycitronellal in pet. Concentration not reported.	24/940 (2.6%) patients were positive.	A retrospective study on patch test data from Department of Dermatology, University Hospital St Rafaël, Belgium (1990-2011).	Nardelli et al. (2013).
Patch test: Prospective study of 100 selected patients with contact allergy patch tested with 7-hydroxycitronellal in pet. Concentration not reported.	8/100 (8%, 95% CI: 3.52-15.16%) patients were positive.	Single-centre, double-blind prospective experimental longitudinal volunteer study at the department of Dermatology of the VU University Medical Centre, The Netherlands (2005-2010).	Nagtegaal et al. (2012).
Patch test: Retrospective study of 1498 consecutive eczema patients patch tested with 7-hydroxycitronellal in 1% pet.	13/1498 (0.9 %) patients were positive.	A retrospective study of patch test data at Department of Dermato-Allergology, Copenhagen University Hospital Gentofte, Denmark (2008-2010).	Heisterberg et al. (2011) and Heisterberg et al. (2012) (corrigendum).
Patch test: Retrospective study of 157 selected patients (chosen out of 509 patients	Ca. 31/157 (ca. 20%) patients were positive.	A retrospective study of patch test data at the Allergy Clinic of the Department of Dermatology and	Turcic et al. (2011).

Method	Results	Remarks/Comments	Reference
positive to fragrance allergens) patch tested with 7-hydroxycitronellal in 1% pet.		Venereology, Zagreb University Hospital Center and School of Medicine, Zagreb, Croatia (2001-2005).	
Patch test: Retrospective study of 86 selected patients patch tested with 7-hydroxycitronellal in 5% pet.	6/86 (7%) patients were positive.	A retrospective and descriptive analysis of a patch test study at the Cutaneous Allergy Unit of a tertiary referral hospital, Spain (2004-2008).	Cuesta et al. (2010).
Patch test: Retrospective study of 1214 consecutive patients and 4359 selected patients patch tested with 7-hydroxycitronellal in 1%.	14/1214 (1.17%, 95% CI: 0.48-1.85%) and 129/4359 (2.95%, 95% CI: 2.43-3.47%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2005-2008).	Uter et al. (2010).
Patch test: Prospective study of 320 selected eczema patients patch tested with 7-hydroxycitronellal in 2% pet.	7/320 (2.2%) patients were positive.	A prospective analysis of selected eczema patients at the University Medical Center in Groningen, the Netherlands (2005-2007).	van Oosten et al. (2009).
Patch test: Retrospective study of 153 selected patients with a) current allergic dermatitis or b) past allergic dermatitis patch tested with 7-hydroxycitronellal in 1% pet.	a) 41/153 (26.8%) and b) 49/153 (32.03%) patients were positive.	A retrospective study of patch test data from patients attending the Department of Cutaneous Allergy at St John's Institute of Dermatology, UK (1982-2007).	White (2009).
Patch test: Prospective study of 15 selected patients with eczematous reactions from ketoprofen-containing gels patch tested with	0/15 (0%) patients were positive.	A prospective study on patch test data from patients from Italy (2006-2007).	Foti et al. (2008).

Method	Results	Remarks/Comments	Reference
7-hydroxycitronellal in 2% pet.			
Patch test: Retrospective study of 2063 consecutive patients patch tested with 7- hydroxycitronellal in 1% pet.	27/2063 (1.3%, 95% CI: 0.7- 1.8%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (2003- 2004).	Schnuch et al. (2007).
Patch test: Retrospective study of a) 33 patients positive to their own deodorant and b) 204 negative to their own deodorant patch tested with 7- hydroxycitronellal in 1% pet.	a) 4/33 (12%) and b) 9/204 (4.4%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (1998- 2002).	Uter et al. (2007).
Patch test: Retrospective study of a) 31 patients positive to their own shaving product/eau de toilette/perfume and b) 210 negative to their own shaving product/eau de toilette/perfume, patch tested with 7-hydroxycitronellal in 1% pet.	a) 4/31 (13%) and b) 4/210 (2%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (1998- 2002).	Uter et al. (2007).
Patch test: study of 30 selected patients with a positive patch test to their own perfumed product patch tested with hydroxycitronellal. Concentration and vehicle not reported.	11/30 (35%) patients were positive.		Vocanson et al. (2006).
Patch test: Retrospective study	127/4900 (2.6%) patients were	A retrospective study on patch test data	Schnuch et al. (2002).

Method	Results	Remarks/Comments	Reference
of 4900 unselected patients patch tested with 7-hydroxycitronellal in 1% pet.	positive. In addition, 566 patients had a + reaction to FM and 46 (8%) of them were positive to hydroxycitronellal 1 % pet. 425 patients had ++/+++ reaction to FM and 77 (18%) were positive to hydroxycitronellal 1 % pet.	from multicentre project IVDK (1996-1999).	
Patch test: Retrospective study of 160 selected patients sensitive to FM patch tested with 7-hydroxycitronellal. Concentration and vehicle not reported.	4/160 (2.5%) patients were positive.	A retrospective study on patch test data from the seven members of the Hungarian Contact Dermatitis Research Group (1998-1999).	Temesvari et al. (2002).
Patch test: prospective study of 747 selected patients with suspected fragrance allergy patch tested with 7-hydroxycitronellal 1% in pet.	11/747 (1.5%) patients were positive.	A prospective analysis of patients from FAZ-Floridsdorf Allergy Centre, Austria (1997-2000).	Wohrl et al. (2001).
Patch test: Study of 226 selected patients sensitive to FM patch tested with 7-hydroxycitronellal 1% in pet.	15/226 (6.6%) patients were positive.	Department of Dermatology, University Hospital, Coimbra, Portugal (1989-1999).	Brites et al. (2000).
Patch test: Study of 1483 selected patients with suspected cosmetic dermatitis patch tested with 7-hydroxycitronellal in 5% pet.	15/1483 (1%) patients were positive.	Nagoya, Japan (year not stated).	Sugiura et al., 2000 cited from SCCS (2012).

Method	Results	Remarks/Comments	Reference
Patch test: Retrospective study of 50 patients sensitive to FM patch tested with 7-hydroxycitronellal 2% in 1% sorbitan sesquioleate.	10/50 (20%) patients were positive.	Retrospective study of patch test data. University Hospital Utrecht, The Netherlands (1994-1998).	Hendriks and van Ginkel (1999).
Patch test: Retrospective study of 40 patients sensitive to FM patch tested with 7-hydroxycitronellal in pet. Concentration not reported.	1/40 (2.5%) patients were positive.		Katsarma and Gawkrödger (1999).
Patch test: Study of 11 patients with perfume allergy patch tested with 7-hydroxycitronellal. Concentration and vehicle not reported.	6/11 (55%) patients were positive.	The patients' cosmetic products were subjected to chemical analysis. The content of 7-hydroxycitronellal was at average 5 times higher in cosmetics from 7-hydroxycitronellal sensitive patients compared with 7-hydroxycitronellal negative patients.	Johansen et al., 1996 cited from SCCNFP (1999).
Patch test: study of 167 selected patients suspected of fragrance sensitivity patch tested with 7-hydroxycitronellal 4%. Vehicle not reported.	23/167 (13.8%) patients were positive.		Larsen et al., 1996 cited from SCCNFP (1999).
Patch test: Study of 1072 patients patch tested with 7-hydroxycitronellal 1% in pet.	8/1072 (0.75%) patients were positive.	European multicentre study with 9 different centres.	Frosch et al., 1995 cited from SCCNFP (1999).
Patch test: Retrospective study of 367 selected patients patch tested with 7-	27/367 (7.4%) patients were positive.	Retrospective study of patch test data from Department of Dermatology, Gentofte Hospital, Denmark	Johansen and Menne (1995).

Method	Results	Remarks/Comments	Reference
hydroxycitronellal in 1-2% pet.		(1979-1983 and 1988-1992).	
Patch test: Prospective study of 50 selected patients positive to a fragrance mix patch tested with 7-hydroxycitronellal. Concentration and vehicle not reported.	5/50 (10%) patients were positive.	Retrospective study of patch test data from Department of Dermatology and Venereology, Hungary. Year not stated.	Becker et al. (1994).
Patch test: Prospective study of 61 selected patients positive to a fragrance mix patch tested with 7-hydroxycitronellal 5% in pet.	12/61 (20%) patients were positive. Control tests in 100 patients not allergic to fragrances showed no positive reactions when tested with 7-hydroxycitronellal 5% pet.	Prospective study of patch test data from University of Amsterdam and University of Leiden, The Netherlands (1987).	de Groot et al. (1993).
Patch test: Prospective study of 162 selected patients positive to a fragrance mix patch tested with 7-hydroxycitronellal 1% in pet.	10/162 (6.2%) patients were positive.	Prospective study of patch test data from Dermatologische Klinik und Poliklinik, Germany (1991).	Enders et al. (1989).
Patch test: Study of 78 selected patients sensitive to FM patch tested with 7-hydroxycitronellal 5%. Vehicle not reported.	7/78 (9%) patients were positive.	Multicentre study involving 6 countries. Year not stated.	Wilkinson et al., 1989 cited from SCCNFP (1999).
Patch test: Study of 156 selected patients with pure contact allergy to cosmetic products patch tested with 7-hydroxycitronellal.	6/156 (3.8%) patients were positive.		Broneck et al., 1987 cited from SCCNFP (1999).

Method	Results	Remarks/Comments	Reference
Concentration and vehicle not reported.			
Patch test: Retrospective study of 63 selected patients with dermatitis tested positive to perfume mixture patch tested between 1983 and 1984 with 7-hydroxycitronellal in 5% pet. and 54 selected patients with dermatitis tested positive to perfume mixture patch tested between 1984 and 1985 with 7-hydroxycitronellal in 1% pet.	Between 1983 and 1984 13/63 (21%) and between 1984 and 1985 9/54 (16%) patients were positive.	Retrospective study of patch test data from Istituto Dermatologico Santa Maria e San Gallicano, Italy (1983-1985).	Santucci et al. (1987).
Patch test: Retrospective study of 403 selected patients with cutaneous reactions to cosmetic products patch tested with 7-hydroxycitronellal. Concentration and vehicle not reported.	7/403 (1.7%) patients were positive.	The number of patients is not clearly stated in the article by Adams & Maibach 1985.	Adams and Maibach (1985).
Patch test: Prospective study of 182 selected patients suspected of contact allergy to cosmetics patch tested with 7-hydroxycitronellal 10%. Vehicle not reported.	19/182 (10.5%) patients were positive.		Malten et al.,, 1984 cited from SCCNFP (1999).
Patch test: study of 20 selected perfume allergic	9/20 (45%) patients were positive.		Larsen et al., 1977 cited from SCCNFP (1999).

Method	Results	Remarks/Comments	Reference
patients patch tested with 7-hydroxycitronellal in 4%.			
Patch test and ROAT ¹ : 13 7-hydroxycitronellal-sensitive patients were tested with 10 and 250 mg/kg of 7-hydroxycitronellal in 10% ethanol in a patch test and a ROAT.	10 mg/kg: 1/13 (8%) positive and 250 mg/kg: 5/13 (38%) positive. Vehicle control: 4/13 (31%) positive.		Heydorn et al., 2003 cited from SCCS (2012).
Patch test and ROAT ¹ : 7 7-hydroxycitronellal-sensitive patients and 7 controls were tested with a dilution series from 4 to 0.00006% (17 steps) of 7-hydroxycitronellal in a patch test and a ROAT.	Step 1: 57% positive, Step 2: 71% positive and Step 3: 100% positive reactions were observed. No positive reactions observed in patch test <0.00012% (0.036 µg/cm ²).		Svedman et al., 2003 cited from SCCNFP (1999).

¹ROAT: Repeated Open Application Test

Table 3 summarises Human Repeat Insult Patch Tests (HRIPTs) and Human Maximisation Tests (HMTs) with 7-hydroxycitronellal.

Table 3. HRIPT and HMT studies with 7-hydroxycitronellal.

Method	Results	Remarks/Comments	Reference
Modified HRIPT: 7-hydroxycitronellal concentration: 2.5% Vehicle: 3:1 ethanol:diethyl phthalate (EtOH:DEP).	0/65 (0%) tests were positive.		Ford et al. (1988).
Modified HRIPT: 7-hydroxycitronellal concentration: 5% Vehicle: 3:1 EtOH:DEP.	1/66 (2%) tests were positive.		Ford et al. (1988).
Modified HRIPT: 7-hydroxycitronellal concentration: 7.5% Vehicle: 3:1	1/66 (2%) tests were positive.		Ford et al. (1988).

Method	Results	Remarks/Comments	Reference
EtOH:DEP.			
Modified HRIPT: 7-hydroxycitronellal concentration: 2.5 or 5% Vehicle: 3:1 EtOH:DEP.	33/100 (33%) tests were positive.	The test was performed in 100 of the subjects that had completed the three HRIPTs described directly above.	Ford et al. (1988).
HMT: 7-hydroxycitronellal concentration: 5% Vehicle: petrolatum (pet.).	0/26 (0%) tests were positive.		Unpublished data (Epstein 1976) cited from Ford et al. (1988).
HMT: 7-hydroxycitronellal concentration: 5% Vehicle: pet.	0/25 (0%) tests were positive.	Males only	Unpublished data (Kligman 1973) cited from Ford et al. (1988).
HMT: 7-hydroxycitronellal concentration: 10% Vehicle: pet.	2/25 (8%) tests were positive.		Unpublished data (Kligman 1976) cited from Ford et al. (1988).
HMT: 7-hydroxycitronellal concentration: 10% Vehicle: pet.	0/25 (0%) tests were positive.		Unpublished data (Kligman 1976) cited from Ford et al. (1988).
HMT: 7-hydroxycitronellal concentration: 12% Vehicle: pet.	4/27 (15%) tests were positive.		Unpublished data (Epstein 1978) cited from Ford et al. (1988).
HMT: 7-hydroxycitronellal concentration: 12% Vehicle: pet.	0/25 (0%) tests were positive.		Unpublished data (Kligman 1978) cited from Ford et al. (1988).
HMT: 7-hydroxycitronellal concentration: 12% Vehicle: pet.	7/26 (27%) tests were positive.		Unpublished data (Epstein 1979) cited from Ford et al. (1988).
HMT: 7-hydroxycitronellal concentration: 12% Vehicle: pet.	6/26 (23%) tests were positive.		Unpublished data (Epstein 1979) cited from Ford et al. (1988).
HMT: 7-hydroxycitronellal concentration: 12% Vehicle: pet.	0/25 (0%) tests were positive.	7-hydroxycitronellal purified from α -pinene	Unpublished data (Kligman 1979) cited from Ford et al. (1988).
HMT:	3/25 (12%)	7-hydroxycitronellal	Unpublished data

Method	Results	Remarks/Comments	Reference
7-hydroxycitronellal concentration: 12% Vehicle: pet.	tests were positive.	purified from <i>d</i> -stereoisomer	(Kligman 1979) cited from Ford et al. (1988).
HMT: 7-hydroxycitronellal concentration: 12% Vehicle: pet.	0/25 (0%) tests were positive.	7-hydroxycitronellal purified from <i>l</i> -stereoisomer	Unpublished data (Kligman 1979) cited from Ford et al. (1988).
HMT: Pseudo 7 -hydroxycitronellal concentration: 12% Vehicle: pet.	1/25 (4%) tests were positive.		Unpublished data (Kligman 1979) cited from Ford et al. (1988).
HMT: 7-hydroxycitronellal concentration: 12% Vehicle: DEP.	2/22 (9%) tests were positive.	Lower boiling point fraction	Unpublished data (Epstein 1980) cited from Ford et al. (1988).
HMT: 7-hydroxycitronellal concentration: 12% Vehicle: DEP.	1/26 (4%) tests were positive.	Higher boiling point fraction	Unpublished data (Epstein 1980) cited from Ford et al. (1988).
HMT: 7-hydroxycitronellal concentration: 12% Vehicle: DEP.	2/21 (10%) tests were positive.	According to Ford et al 1988 7-hydroxycitronellal was "Tested on same panel with washed cinnamic alcohol."	Unpublished data (Epstein 1980) cited from Ford et al. (1988).

HR IPT: Human Repeat Insult Patch Test, HMT: Human Maximisation Test.

Case studies

Table 4 summarises case reports with ACD where 7-hydroxycitronellal has been found to be among the causative agents.

Table 4. Case studies with 7-hydroxycitronellal.

Method	Results	Remarks/Comments	Reference
Patch test: A 48-year old male metalworker with recurrent hand dermatitis was patch tested with 7-hydroxycitronellal and a long list of other allergens.	7-hydroxycitronellal and several other allergens tested positive.	Case study (Germany, 2007-2008).	Tanko et al. (2009).
Patch test: A 52-year old man with contact allergy to his after-shave was	Positive reaction was observed.	Case study, (year and country not stated).	De Groot and Liem 1983 cited from SCCNFP

Method	Results	Remarks/Comments	Reference
patch tested with 7-hydroxycitronellal. Concentration and vehicle not reported.			(1999).
Patch test: A 32-year old barber with hand eczema was patch tested with 10% 7-hydroxycitronellal in petrolatum.	Positive reactions to 7-hydroxycitronellal, methyl 2-octynoate and cinnamyl alcohol were observed.	Case study, (year and country not stated).	Van Ketel 1978 cited from SCCNFP (1999).

A total of 39 results from patch test population studies, 4 modified HRIPTs, 15 HMTs and 3 case studies with 7-hydroxycitronellal are summarised above (Table 2, 3 and 4). As shown in Table 2 the positive patch test frequencies from all of the reported patch test population studies vary between 0 and 55% in dermatitis patients. For unselected/consecutive dermatitis patients, positive reactions range between 0.9 and 2.6% (4 studies) and for selected dermatitis patients positive reactions range between 0 and 55% (35 studies). The total number of published cases is > 800. Sensitisation was reported in 3/4 modified HRIPT studies at 7-hydroxycitronellal concentrations from 2.5 to 7.5% but without dose-response. In the HMT studies, sensitisation was reported in 0/2 tests with 5% 7-hydroxycitronellal, 1/2 tests with 10% 7-hydroxycitronellal and in 8/11 tests with 12% 7-hydroxycitronellal. Based on these data the Research Institute for Fragrance Materials, Inc. (RIFM) deducted a NOEL-HRIPT¹⁶ (induction) of 5000 µg/cm² and a LOEL-HRIPT/HMT¹⁷ (induction) of 5906 µg/cm². In addition, based on weight of evidence, a No Expected Sensitization Induction Level (NESIL) of 5000 µg/cm² was established for 7-hydroxycitronellal by the RIFM Expert Panel (IFRA, 2013c).

According to SCCS (2012) 7-hydroxycitronellal is used in volumes less than 175 ton per year in perfume formulations. It has been reported that in consumer products containing fragrance allergens that are required to be labelled 10.8% of a total of 516 consumer products; 17% of a total of 300 consumer products; ca. 8% of 3000 products and 6.3% of children's cosmetics were labelled to contain 7-hydroxycitronellal (Wijnhoven et al., 2008; Buckley, 2007; Schnuch et al., 2009 and Poulsen & Schmidt, 2007 cited from SCCS (2012)). In addition, in 2007, 27.3% of 88 tested deodorants were labelled to contain 7-hydroxycitronellal and the fragrance was detected in 70% (range: 1-1746 mg/kg) of 23 deodorants selected for analysis (Rastogi et al., 2007 cited from SCCS (2012)).

The IFRA standard limits for 7-hydroxycitronellal in different IFRA QRA product categories reported by IFRA (2013c and 2015) are shown in table 5.

¹⁶ NOEL-HRIPT: No Observed Effect Level-Human Repeat Insult Patch Test.

¹⁷ LOEL-HRIPT/HMT: Lowest Observed Effect Level-Human Repeat Insult Patch Test/Human Maximisation test.

Table 5. The IFRA standard limits for 7-hydroxycitronellal in IFRA QRA product categories.

IFRA category	QRA product	Product type that drives the category consumer exposure level	IFRA standard limits
Category 1		Lip products	0.1%
Category 2		Deodorants/antiperspirants	0.2%
Category 3		Hydroalcohols for shaved skin	0.8%
Category 4		Hydroalcohols for unshaved skin	1.0%
Category 5		Hand cream	1.0%
Category 6		Mouthwash	3.6%
Category 7		Intimate wipes	0.4%
Category 8		Hair styling aids	1.0%
Category 9		Rinse-off hair conditioners	1.0%
Category 10		Hard surface cleaners	1.0%
Category 11		Candles	Not restricted

IFRA: International Fragrance Association, QRA: Quantitative Risk Assessment.

7-Hydroxycitronellal is registered under the REACH regulation with an annual tonnage band of 100 - 1000 tonnes per annum.

Summary and discussion of skin sensitization

Human data

A total of 39 results from patch test population studies, 4 modified HRIPTs, 15 HMTs and 3 case studies were identified with 7-hydroxycitronellal. The positive patch test frequencies from all of the reported patch test population studies vary between 0 and 55% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0.9 and 2.6% (4 studies) and in studies with selected dermatitis patients positive reactions range between 0 and 55% (35 studies). The total number of published cases is > 800. A LOEL-HRIPT/HMT (induction) of 5906 µg/cm² was established for 7-hydroxycitronellal by the RIFM Expert Panel.

Non-human data

A total of 7 LLNAs including 1 LLNA *ex vivo* BrdU, 1 GPMT and 1 Buehler test were identified testing skin sensitising effects of 7-hydroxycitronellal. The reported EC3 values for 7-hydroxycitronellal range between 9.8 and 33%. In the GPMTs sensitisation was observed but not quantified (i.e. number of animals affected) in 3/6 studies with intradermal induction doses of 0.4, 5 and 10% citral. In the GPMT sensitisation in 60% of the animals (number of animals not reported) after an intradermal induction dose of 0.5% 7-hydroxycitronellal. Sensitisation was also observed in 38% of the animals in the Buehler test with an induction concentration of 30% 7-hydroxycitronellal.

No relevant *in vitro* studies on 7-hydroxycitronellal (i.e. OECD TG 442C and OECD TG 442D) were identified in the literature.

Exposure

According to data from IFRA (2013c) the exposure of 7-hydroxycitronellal when used as fragrance in cosmetics and in other consumer products appears to be low.

Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 0.9 and 2.6% with 1/4 studies reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 0 and 55% with 29 out of 33 studies reporting frequencies equal to or higher than 2%. In addition to this there are more than 800 published cases of positive patch test reactions to 7-hydroxycitronellal. According to the CLP criteria a frequency $\geq 1\%$ for unselected/consecutive dermatitis patients and/or $\geq 2\%$ for selected dermatitis patients and/or a total number of published cases ≥ 100 , equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b). The collected data described above from patch test studies show that 7-hydroxycitronellal causes a *high frequency* of occurrence of skin sensitisation based on these three types of information. In regard to the HRIPT/HMT data the positive response reported at $> 500 \mu\text{g}/\text{cm}^2$ for HRIPT/HMT induction threshold indicate evidence for sub-category 1B according to Annex I: 3.4.2.2.2.2.

In the seven LLNAs EC3 values between 9.8 and 33% were reported for 7-hydroxycitronellal. According to the CLP Regulation an EC3 value $\leq 2\%$ indicates classification of a substance in sub-category 1A whereas an EC3 value $> 2\%$ indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.). Thus, all seven studies indicate classification of 7-hydroxycitronellal in sub-category 1B.

In the GPMT sensitisation was observed in 60% of the animals after an intradermal induction dose of 0.5% 7-hydroxycitronellal. According to the CLP criteria a positive response $\geq 60\%$ of the animals responding at $>0.1\%$ to $\leq 1\%$ intradermal induction dose indicates classification of a substance in sub-category 1A (Annex I: 3.4.2.2.3.2.) and thus, this study indicates classification of 7-hydroxycitronellal into sub-category 1A.

Sensitisation was also observed in 38% of the animals in a Buehler test with an induction concentration of 30% 7-hydroxycitronellal. According to the CLP criteria a positive response $\geq 15\%$ of the animals responding at $>20\%$ topical induction dose indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.3.) and thus, this study indicates classification of 7-hydroxycitronellal into sub-category 1B.

Overall, there is clear evidence for classification in sub-category 1A based on the human patch test studies showing a high frequency of occurrence of skin sensitisation and the total number of cases combined with the estimated low exposure. Except from the GMTP study, which supports a sub-category 1A classification, the remaining animal studies (LLNA and Buehler) indicate a classification in sub-category 1B. A classification as a skin sensitizer in sub-category 1A is thus warranted for 7-hydroxycitronellal.

Conclusions on classification and labelling

Based on the high frequency of sensitisation observed in human patch test studies and the high number of published cases combined with the estimated low exposure a classification of 7-hydroxycitronellal as a skin sensitizer in sub-category 1A is justified.

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Appendix 12 Methyl oct-2-ynate CAS RN 111-12-6

Non-human information

Table 1 summarises relevant animal studies with methyl oct-2-ynate i.e. Local Lymph Node Assays (LLNAs), Guinea Pig Maximization Tests (GPMTs) and Buehler tests.

Table 1. Animal studies with methyl oct-2-ynate.

Method	Results	Remarks/Comments	Reference
LLNA, according to OECD test guideline 429.	Methyl oct-2-ynate was shown to have an EC3 value of 0.45%.	The reaction mechanistic domain was reported to be the Michael receptor.	Unpublished report by RIFM 2006 cited from Kern et al. (2010).
LLNA, according to OECD test guideline 429. 0.5, 1, 2, 5 and 10% methyl oct-2-ynate. Vehicle not reported.	Methyl oct-2-ynate was shown to have an EC3 value of <0.5% (<0.032 M).	Methyl oct-2-ynate should also have been tested at lower concentrations.	Unpublished report by RIFM 2005k cited from SCCS (2012).
GPMT, intradermal induction 0.625, 5 and 10%; topical induction 1, 3 and 30%; challenge 0.3, 0.9 and 3% methyl oct-2-ynate. Vehicle not reported.	18/20 (90%) at the least severe and middle regimens and 20/20 (100%) at the most severe.	No further information was available from Hostynek and Maibach (2006).	Unpublished report by Buehler et al., 1985 cited from Hostynek and Maibach (2006).
Buehler test, induction dose 2.5% with challenge doses of 0.5, 1.5 and 5% methyl oct-2-ynate. Vehicle not reported.	Positive reactions in 9/20 (45%), 12/20 (60%) and 14/20 (70%) at challenge doses of 0.5, 1.5 and 5% methyl oct-2-ynate, respectively.	No further information was available from Hostynek and Maibach (2006).	Unpublished report by Buehler et al., 1986 cited from Hostynek and Maibach (2006).

A total of 2 LLNAs (OECD TG 429), 1 GPMT and 1 Buehler test with methyl oct-2-ynate are summarised in table 1. In both LLNA studies an EC3 value of <0.5% was reported. In the GPMT sensitisation was observed in 90 % of the animals after an intradermal induction dose of 0.625% methyl oct-2-ynate. Sensitisation was also observed in the Buehler test with positive reactions in 45-70% of the animals after an induction dose of 2.5%

According to the review by Hostynek and Maibach (2006) several other (mostly unpublished) animal tests including open and closed epicutaneous tests, Draize tests, a Maguire test, a Freund's Complete Adjuvant Test and an additional GPMT testing the sensitising potential of methyl oct-2-ynate exist. However, these tests were either not relevant for the purpose of sub-categorisation or considered by Hostynek and Maibach (2006) to be of poor quality and are not reported here.

No relevant *in vitro* studies on methyl oct-2-ynate (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

Human Studies

Population studies

Table 2 summarises patch test studies on methyl oct-2-ynate involving several thousand dermatitis patients from various countries in Europe and the US. Most of the studies are diagnostic patch test studies.

Table 2. Population studies with methyl oct-2-ynate.

Method	Results	Remarks/Comments	Reference
Patch test: Retrospective study of 1870 patients patch tested with methyl oct-2-ynate. Concentration and vehicle not reported.	3/1870 (0.16%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2007-2009).	Schnuch et al. (2015).
Patch test: Retrospective and descriptive study of 1951 eczema patients patch tested with methyl oct-2-ynate 1% in petrolatum (pet.).	3/1951 (0.15%, 95% CI: 0.0-0.3%) patients were positive.	A retrospective study on patch test data at St John's Institute of Dermatology at St Thomas' Hospital, UK (2011-2012).	Mann et al. (2014).
Patch test: Retrospective and descriptive study on 211 eczema patients tested with methyl oct-2-ynate 1% in pet.	1/211 (0.5%) patients were positive. Active sensitisation was observed in two patients and the testing was stopped.	Retrospective descriptive analysis of a patch test study at Gentofte Hospital, Denmark (2008-2010).	Heisterberg et al. (2011).
Patch test: Study of 230 consecutive eczema patients patch tested with methyl oct-2-ynate 1% in pet.	0/230 (0%) patients were positive.	Retrospective descriptive analysis of a patch test study at Gentofte Hospital, Denmark (2007-2008).	Heisterberg (2010).
Patch test: Study of 120 consecutive	2/120 (1.67%) patients were	Retrospective descriptive analysis of	Heisterberg (2010).

Method	Results	Remarks/Comments	Reference
eczema patients patch tested with methyl oct-2-ynate 2% in pet.	positive.	a patch test study at the department of Dermatologie at CHU Saint Jacques, France.	
Patch test: Study of 988 selected patients tested with methyl oct-2-ynate 1% in pet.	1/988 (0.1%, 95% CI: 0-0.2%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2005-2008).	Uter et al. (2010).
Patch test: Study of 320 selected eczema patients patch tested with methyl oct-2-ynate 0.5% in pet.	1/320 (0.3%) patients were positive.	Retrospective analysis of a patch test study at the University Medical Center in Groningen, the Netherlands (2005-2007).	van Oosten et al. (2009).
Patch test: Study of 2401 unselected patients patch tested with methyl oct-2-ynate 1% in pet.	6/2401 (0.2%, 95% CI: 0.0-0.4%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2003-2004).	Schnuch et al. (2007).
Patch test: Study of 182 patients suspected of contact allergy to cosmetics patch tested with 0.5% methyl oct-2-ynate. Vehicle not reported.	2/182 (1.1%) patients were positive.		Unpublished report by Malten et al., 1984 cited from SCCNFP (1999).
Patch test: Study of 34 patients with allergic contact dermatitis to cosmetics patch tested with 0.5% methyl oct-2-ynate. Vehicle not reported.	1/34 (2.9%) patients were positive.	Pilot study performed prior to the study described directly above.	Unpublished report by Malten et al., 1984 cited from SCCNFP (1999).
Patch test: Study of 278 patients patch tested with 1% methyl oct-2-	1/278 (0.4%) patients were positive.	A study performed by the North American Contact Dermatitis Research Group.	Unpublished report by Mitchell et al., 1982 cited from SCCNFP

Method	Results	Remarks/Comments	Reference
ynate. Vehicle not reported.			(1999).

Case studies

Table 3 summarises case reports with allergic contact dermatitis in different clinics in Europe where methyl oct-2-ynate has been found as a causative agent.

Table 3. Case studies with methyl oct-2-ynate.

Method	Results	Remarks/Comments	Reference
Patch test: A 42-year old woman under investigation for postsurgical ¹ ACD and with a previous reaction towards deodorants was patch tested with 2% methyl oct-2-ynate in petrolatum (pet.).	Delayed positive reaction on D16 was observed.	Case study, France (year not stated).	Heisterberg (2010).
Patch test: A 28-year old woman with facial eczema was tested and repeat tested with 1% methyl oct-2-ynate in pet.	Delayed positive reaction on D20 and on D2 (1+) in the repeat test was observed.	Case study, Denmark (year not stated).	Heisterberg (2010).
Patch test: A 21-year old woman suspected of occupational hand eczema was patch tested and repeat tested with 1% methyl oct-2-ynate in pet.	Delayed positive reaction 4 weeks after first test and positive reaction on D2 (2+) in the repeat test was observed.	Case study, Denmark (year not stated).	Heisterberg (2010).
Patch test: A 19-year old woman with work-related ¹ ACD was patch tested with 1% methyl oct-2-ynate in	Positive reaction on D2 was observed.	Case study, UK (1985).	English and Rycroft (1988).

Method	Results	Remarks/Comments	Reference
methyl ethyl ketone.			
Patch test: A 32-year old man with hand eczema was patch tested with 0.5% methyl oct-2-ynate in pet.	Positive reactions to methyl oct-2-ynate, 7-hydroxycitronellal and cinnamyl alcohol were observed.	Case study, (year and country not stated).	Van Ketel (1978) cited from SCCNFP (1999).

^aACD: Allergic Contact Dermatitis.

A total of 11 results from patch test population studies and 5 cases with methyl oct-2-ynate are summarised above (Table 2 and 3). As shown in Table 2 the positive patch test frequencies from all of the reported patch test population studies vary between 0 and 2.9% in dermatitis patients. For unselected/consecutive dermatitis patients, positive reactions range between 0 and 1.67% (3 studies) and for selected dermatitis patients positive reactions range between 0.1 and 2.9% (8 studies). The total number of published cases is > 25. The Research Institute for Fragrance Materials, Inc. (RIFM) deducted a NOEL-HRIPT¹⁸ (induction) of 118 µg/cm² and a LOEL-HRIPT/HMT¹⁹ (induction) of 194 µg/cm². In addition, based on weight of evidence, a No Expected Sensitization Induction Level (NESIL) of 110 µg/cm² was established for methyl oct-2-ynate by the RIFM Expert Panel (IFRA, 2008d).

One of the case studies reports three individual cases (table 3) (Heisterberg, 2010). In these cases late patch test reactions (after 2-4 weeks) were observed. Positive repeat tests in two of these cases were reported indicating active sensitization (i.e. the subjects were sensitized by the patch test). The third case was not repeat patch tested.

SCCS (2012) describes methyl oct-2-ynate as an extreme but rare allergen.

According to SCCS (2012) methyl oct-2-ynate is used in volumes less than 175 ton per year in perfume formulations. It has been reported that in consumer products containing fragrance allergens that are required to be labelled 1.1% of 88 tested deodorants (in 2007), 1% of a total of 516 consumer products; 0% of a total of 300 consumer products; ca. 0.5% of 3000 products and 0% of children's cosmetics were labelled to contain citral (Wijnhoven et al., 2008; Buckley, 2007; Schnuch et al., 2009 and Poulsen & Schmidt, 2007 cited from SCCS (2012)). Based on these data SCCS (2012) concluded that methyl oct-2-ynate was among the least used fragrance ingredients in cosmetics and other consumer products.

The IFRA standard limits for methyl oct-2-ynate in different IFRA QRA product categories reported by IFRA (2008d and 2015) are shown in table 4.

¹⁸ NOEL-HRIPT: No Observed Effect Level-Human Repeat Insult Patch Test.

¹⁹ LOEL-HRIPT/HMT: Lowest Observed Effect Level-Human Repeat Insult Patch Test/Human Maximisation test.

Table 4. The IFRA standard limits for methyl oct-2-ynate in IFRA QRA product categories.

IFRA category	QRA product	Product type that drives the category consumer exposure level	IFRA standard limits
Category 1		Lip products	0.003%
Category 2		Deodorants/antiperspirants	0.004%
Category 3		Hydroalcohols for shaved skin	0.01%
Category 4		Hydroalcohols for unshaved skin	0.01%
Category 5		Hand cream	0.01%
Category 6		Mouthwash	0.08%
Category 7		Intimate wipes	0.008%
Category 8		Hair styling aids	0.01%
Category 9		Rinse-off hair conditioners	0.01%
Category 10		Hard surface cleaners	0.01%
Category 11		Candles	Not restricted

IFRA: International Fragrance Association, QRA: Quantitative Risk Assessment.

Methyl oct-2-ynate is not registered under the REACH regulation.

Summary and discussion of skin sensitization

Human data

A total of 11 results from patch test population studies and 5 case studies were identified with methyl oct-2-ynate. The positive patch test frequencies from all of the reported patch test population studies vary between 0 and 2.9% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 0 and 1.67% (3 studies) and in studies with selected dermatitis patients positive reactions range between 0.1 and 2.9% (8 studies). The total number of published cases is > 25. A LOEL-HRIPT/HMT (induction) of 194 µg/cm² was established for methyl oct-2-ynate by the RIFM Expert Panel based on unpublished reports.

Non-human data

A total of 2 LLNAs (OECD TG 429), 1 GPMT and 1 Buehler test were identified testing skin sensitising effects of methyl oct-2-ynate. In both LLNA studies an EC3 value of <0.5% was reported. In the GPMT sensitisation was observed in 90 % of the animals after an intradermal induction dose of 0.625% methyl oct-2-ynate. Sensitisation was also observed in the Buehler test with positive reactions in 45-70% of the animals after an induction dose of 2.5%.

No relevant *in vitro* studies on methyl oct-2-ynate (i.e. OECD TG 442C and OECD 442D) were identified in the literature.

Exposure

According to data from IFRA (2008d) the exposure of methyl oct-2-ynate when used as fragrance in cosmetics and in other consumer products appears to be low.

Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 0 and 1.67% with 1/3 studies reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 0.1 and 2.9% with 1 out of 8 studies reporting frequencies higher than 2%. In addition to this there are more than 25 published cases of positive patch test reactions to methyl oct-2-ynate. According to the CLP criteria a frequency $\geq 1\%$ for unselected/consecutive dermatitis patients and/or $\geq 2\%$ for selected dermatitis patients and/or a total number of published cases ≥ 100 , equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that methyl oct-2-ynate causes a *low/moderate frequency* of occurrence of skin sensitisation based on these three types of information.

In regard to HRIPT studies positive responses were observed at exposure to 194 $\mu\text{g}/\text{cm}^2$ methyl 2-octynoate. A positive response at $\leq 500 \mu\text{g}/\text{cm}^2$ in a HRIPT or HMT suggests categorisation into sub-category 1A according to Annex I: 3.4.2.2.2.1 and 3.4.2.2.2.2.

In the 2 LLNAs EC3 values were $<0.5\%$. According to the CLP Regulation an EC3 value $\leq 2\%$ indicates classification of a substance in sub-category 1A (Annex I: 3.4.2.2.3.2.).

In the GPMT sensitisation was observed in 90% of the animals after an intradermal induction dose of 0.625%. According to the CLP criteria a positive response $\geq 60\%$ of the animals responding at $> 0.1\%$ to $\leq 1\%$ intradermal induction dose indicates classification of a substance in sub-category 1A (Annex I: 3.4.2.2.3.2.).

In the Buehler test sensitisation was observed in 45-70% of the animals after an induction dose of 2.5%. According to the CLP criteria a positive response $\geq 60\%$ of the animals responding at $> 0.2\%$ to $\leq 20\%$ topical induction dose indicates classification of a substance in sub-category 1A (Annex I: 3.4.2.2.3.2.).

Overall, there is clear evidence for classification in sub-category 1A based on the very low EC3 values from the LLNAs. The results from GPMT and the Buehler test also supports sub-category 1A. Data from human patch test studies and the number of published cases justify classification of methyl oct-2-ynate in sub-category 1B while data from HRIPT studies justify classification of methyl oct-2-ynate in sub-category 1A. A classification as a skin sensitiser in sub-category 1A is thus warranted for methyl oct-2-ynate.

Conclusions on classification and labelling

Based on HRIPT data, the very low EC3 value from LLNAs and results from GPMT and Buehler tests a classification of methyl oct-2-ynate as a skin sensitiser in sub-category 1A is justified.

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Appendix 13 *Cinnamomum cassia* leaf oil and *Cinnamomum zeylanicum*, ext. CAS RN 84961-46-6/8007-80-5 and 84649-98-9

Cinnamomum cassia leaf oil and *Cinnamomum zeylanicum* ext. are not specifically defined chemical substances but oils and extracts of leaves, twigs, wood, bark or the whole plant of *Chinese Cinnamon Cinnamomum cassia* (L.), *Lauraceae* and *Ceylon Cinnamon, Cinnamomum zeylanicum*, *Lauraceae*, respectively.

Searching for the CAS RN for *Cinnamomum cassia* leaf oil (84961-46-6 / 8007-80-5) in the European Cosmetic ingredient database CosIng yields a total of seven INCI names (Table 1).

Table 1. *Cinnamomum cassia* leaf oil.

CAS RN	EC No	INCI Name	Description
84961-46-6 / 8007-80-5	284-635-0 / -	<i>Cinnamomum cassia</i> leaf oil	"Cassia Oil"; "Cassia leaf Oil"; "Cinnamon Oil Chinense". <i>Cinnamomum Cassia</i> Leaf Oil is the volatile oil obtained by steam distillation from the leaves and twigs of the Chinese Cinnamon, <i>Cinnamomum cassia</i> (L.), <i>Lauraceae</i> .
		<i>Cinnamomum cassia</i> bark	<i>Cinnamomum Cassia</i> Bark is a plant material derived from the dried bark of the Chinese Cinnamon, <i>Cinnamomum cassia</i> (L.), <i>Lauraceae</i> .
		<i>Cinnamomum cassia</i> bark extract	<i>Cinnamomum Cassia</i> Bark Extract is an extract obtained from the dried bark of the Chinese Cinnamon, <i>Cinnamomum cassia</i> (L.), <i>Lauraceae</i> .
		<i>Cinnamomum cassia</i> extract	<i>Cinnamomum Cassia</i> Extract is the extract of the whole plant , <i>Cinnamomum cassia</i> , <i>Lauraceae</i> .
		<i>Cinnamomum cassia</i> leaf extract*	<i>Cinnamomum Cassia</i> Leaf Extract is an extract obtained from the leaves of the Chinese Cinnamon, <i>Cinnamomum cassia</i> (L.), <i>Lauraceae</i> .
		<i>Cinnamomum cassia</i> oil	<i>Cinnamomum Cassia</i> Oil is the volatile oil obtained from the whole plant of the Chinese Cinnamon, <i>Cinnamomum cassia</i> (L.), <i>Lauraceae</i> .
		<i>Cinnamomum cassia</i> wood extract*	<i>Cinnamomum Cassia</i> Wood Extract is an extract obtained from the wood of the Chinese Cinnamon, <i>Cinnamomum cassia</i> (L.), <i>Lauraceae</i> .

*According to the Cosmetic Directive not an INCI name but Perfuming Name
(<http://ec.europa.eu/growth/tools-databases/cosing/index.cfm?fuseaction=search.results>).

The constituents of Cassia bark extract and Cassia oil and their concentrations are reported reported in Annex I to the IFRA Standards (48th Amendment) (<http://www.ifraorg.org/en-us/search/s/84649-98-9#.Vjsc1rcveUk>) and summarized in Table 2.

Table 2. Constituents of *Cinnamomum cassia* leaf oil.

<i>Cinnamomum cassia</i> leaf oil				Constituents		
CAS RN	EC No	Principle Name	Botanical name	CAS RN	Principle name	Level (%)
84961-46-6 / 8007-80-5	284-635-0 / -	Cassia bark extract	<i>Cinnamomum cassia</i> Blume	1504-74-1	o-Methoxycinnamaldehyde	2
				100-52-7	Benzaldehyde	2
				120-51-4	Benzyl benzoate	0.07
				104-55-2	Cinnamaldehyde	44
				104-54-1	Cinnamyl alcohol	0.5
				91-64-5	Coumarin	0.15
				97-53-0	Eugenol	0.03
84961-46-6 / 8007-80-5	284-635-0 / -	Cassia oil	<i>Cinnamomum cassia</i> Blume	1504-74-1	o-Methoxycinnamaldehyde	4
				100-52-7	Benzaldehyde	4
				120-51-4	Benzyl benzoate	0.14
				104-55-2	Cinnamaldehyde	87
				104-54-1	Cinnamyl alcohol	1
				91-64-5	Coumarin	0.3
				97-53-0	Eugenol	0.06

Searching for the CAS RN for *Cinnamomum zeylanicum* bark oil (84649-98-9) in the European Cosmetic ingredient database CosIng yields five INCI names (Table 3).

Table 3. *Cinnamomum zeylanicum* bark.

CAS RN	EC No	INCI Name	Description
84649-98-9	283-479-0	<i>Cinnamomum zeylanicum</i> bark oil	("Cumnamon Bark Oil Ceylon"; "Cinnamon Oil Ceylon". <i>Cinnamomum Zeylanicum</i> Bark Oil is the volatile oil expressed from the bark of the Ceylon Cinnamon, <i>Cinnamomum zeylanicum</i> , <i>Lauraceae</i> . It contains cinnamaldehyde (50-60%), eugenol (4-8%), phellandrene
		<i>Cinnamomum zeylanicum</i> bark extract	<i>Cinnamomum zeylanicum</i> Bark Extract is an extract obtained from the dried bark of the Ceylon Cinnamon, <i>Cinnamomum zeylanicum</i> , <i>Lauraceae</i> .
		<i>Cinnamomum zeylanicum</i> bark powder	<i>Cinnamomum zeylanicum</i> Bark Powder is the powder obtained from the dried, ground bark of the Ceylon Cinnamon, <i>Cinnamomum zeylanicum</i> , <i>Lauraceae</i> .
		<i>Cinnamomum zeylanicum</i> leaf extract	<i>Cinnamomum zeylanicum</i> Leaf Extract is an extract obtained from the leaves of the Ceylon Cinnamon, <i>Cinnamomum zeylanicum</i> , <i>Lauraceae</i> .
		<i>Cinnamomum zeylanicum</i> leaf oil	("Cinnamon Leaf Oil Ceylon". <i>Cinnamomum Zeylanicum</i> Leaf Oil is the volatile oil obtained from the leaves of the Ceylon Cinnamon, <i>Cinnamomum zeylanicum</i> , <i>Lauraceae</i> .

The constituents of Cinnamon bark extract, Cinnamon bark oil and cinnamon leaf oil and their concentrations are reported in Annex I to the IFRA Standards (48th Amendment) (<http://www.ifraorg.org/en-us/search/s/84649-98-9#.Vjsc1rcveUk>) and summarized in Table 4.

Table 4. Constituents of *Cinnamomum zeylanicum* ext.

<i>Cinnamomum zeylanicum</i> ext.				Constituents		
CAS RN	EC No	Principle Name	Botanical name	CAS RN	Principle name	Level (%)
84649-98-9	283-479-0	Cinnamon bark extract	<i>Cinnamomum</i> spp.	100-52-7	Benzaldehyde	0.1
				120-51-4	Benzyl benzoate	0.3
				104-55-2	Cinnamaldehyde	38
				104-54-1	Cinnamyl alcohol	0.1
				91-64-5	Coumarin	0.3
				97-53-0	Eugenol	1
				97-54-1	Isoeugenol	0.01
84649-98-9	283-479-0	Cinnamon bark oil	<i>Cinnamomum</i> spp.	100-52-7	Benzaldehyde	0.26
				120-51-4	Benzyl benzoate	0.66
				104-55-2	Cinnamaldehyde	75
				104-54-1	Cinnamyl alcohol	0.26
				91-64-5	Coumarin	0.66
				97-53-0	Eugenol	2.2
				97-54-1	Isoeugenol	0.02
84649-98-9	283-479-0	Cinnamon leaf oil	<i>Cinnamomum zeylanicum</i> Blume	100-52-7	Benzaldehyde	0.16
				120-51-4	Benzyl benzoate	3.5
				104-55-2	Cinnamaldehyde	2
				91-64-5	Coumarin	0.3
				97-53-0	Eugenol	70
				4602-84-0	Farnesol	0.12

<i>Cinnamomum zeylanicum</i> ext.	Constituents		
	97-54-1	Isoeugenol	0.13
	93-15-2	Methyl eugenol	0.01

In conclusion the actual composition and concentration of compounds varies between *Cinnamomum cassia* leaf oil and *Cinnamomum zeylanicum* ext. and especially according to the source of the oil or extract (e.g. leaves, bark etc.). This complicates the evaluation unless the source is specified e.g. for patch test studies or LLNA. This is reflected in the few relevant studies identified and summarised below.

Non-human information

No animal studies (LLNA, GPMT or Buehler) with *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext. have been identified.

No relevant *in vitro* studies (OECD TG 442C and OECD 442D) with *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext. have been identified.

Human information

Population studies

Table 5 summarises the few patch test studies on *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext. involving less than 300 dermatitis patients.

Table 5. Population studies with *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext.

Method	Results	Remarks/Comments	Reference
Patch test: Study of 86 selected fragrance mix I positive patients patch tested with "cassia" essential oil 2% in petrolatum (pet.).	24/86 (27.9%) patients were positive.		Rudzki & Grzywa 1986 cited from SCCS (2012).
Patch test: Study of 200 patients patch tested with "cassia" essential oil 2% in pet.	2/200 (1%) patients were positive.		Rudzki et al 1976 cited from SCCS (2012).

Case studies

Table 6 shows one case report with allergic contact dermatitis in Spain where *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext. has been found as causative agents.

Table 6. Case studies with *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext.

Method	Results	Remarks/Comments	Reference
Patch test: A 32-year old man with dermatitis was patch tested with "cinnamon oil" 0.5% in petrolatum.	Positive reaction was observed.	Case study, Spain (year not stated).	Sanchez-Perez & Garcia-Diez 1999 cited from SCCS (2012).

As reflected in table 5 and 6 very few human studies with *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext. have been identified. All three studies are cited from SCCS (2012). A total of 27 positive cases and frequencies between 1 and 27.8% in selected dermatitis patients tested with "cassia" essential oil or "cinnamon oil" were observed.

SCCS (2012) considered the "essential oil" as an 'Established contact allergen in humans' considering the content of well-known allergenic compounds.

Cinnamomum zeylanicum, ext. is registered under the REACH regulation with an annual tonnage band of 1000 - 10 000 tonnes per annum.

Summary and discussion of skin sensitization

Human data

A total of 27 positive cases and frequencies between 1 and 27.8% in selected dermatitis patients tested with "cassia" essential oil or "cinnamon oil" were observed.

Non-human data

No animal studies with *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext. have been identified.

Exposure

It has not been possible to identify any data on exposure to *Cinnamomum cassia* leaf oil or *Cinnamomum zeylanicum*, ext.

Comparison with criteria

One out of two studies with "cassia" essential oil gave a frequency of positive patch tests in selected patients of 27.8% i.e. $\geq 2\%$, which indicate categorisation into sub-category 1A.

No animal studies with *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext. have been identified.

Conclusions on classification and labelling

Data on *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext. alone is insufficient for sub-categorisation of *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext. according to CLP criteria. It may be possible to sub-categorise *Cinnamomum cassia* leaf oil/*Cinnamomum zeylanicum*, ext. based on their constituents by read across to the major compounds such as cinnamaldehyde (for Cassia bark extract, Cassia oil, Cinnamon bark extract and Cinnamon bark oil) and eugenol (for Cinnamon leaf oil).

References

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Appendix 14 *Evernia prunastri* ext. CAS RN 90028-68-5

The main sensitizers of *Evernia prunastri* ext. has been identified as atranol (CAS RN 526-37-4) and chloroatranol (CAS RN 57074-21-2) which are degradation products of atranorin and chloratranorin, respectively, and very potent allergens according to the SCCS (2012).

Non-human information

Table 1 summarises relevant animal studies with *Evernia prunastri* ext. i.e. Local Lymph Node Assays (LLNAs), Guinea Pig Maximization tests (GPMTs) and Buehler tests.

Table 1. Animal studies with *Evernia prunastri* ext.

Method	Results	Remarks/Comments	Reference
LLNA: <i>Ex vivo</i> BrdU. 0.5, 1, 5 and 10% <i>Evernia prunastri</i> ext. Vehicle: 4:1 Acetone:Olive oil.	<i>Evernia prunastri</i> ext. was shown to have an EC3 value of 3.4%.		Ulker et al. (2014).
LLNA: 2.5, 5, 10, 25 and 50% <i>Evernia prunastri</i> ext. Vehicle: 1:3 ethanol:diethyl phthalate.	<i>Evernia prunastri</i> ext. was shown to have an EC3 value of 3.9%.	According to SCCS (2012) there were no reported deviations from OECD TG 429.	Unpublished summary report by RIFM 2009 (RIFM 2004j) cited from SCCS (2012).
GPMT: Intradermal induction 20%; Topical induction 5% and Challenge dose 0.1, 0.3 and 1% <i>Evernia prunastri</i> ext. Vehicle: not reported.	0/8 (0%), 2/8 (25%) and 5/8 (63%) animals were sensitised at challenge doses of 0.1, 0.3 and 1% <i>Evernia prunastri</i> ext., respectively.		Ehret et al., 1992 cited from SCCNFP (2000).

A total of 1 LLNA, 1 LLNA *ex vivo* BrdU and 1 GPMT with *Evernia prunastri* ext. are summarised in table 1. The reported EC3 value for *Evernia prunastri* ext. was 3.9% in the LLNA and 3.4% in the LLNA *ex vivo* BrdU. In the GPMT sensitisation was observed in 5/8 (63%) animals after an intradermal induction dose of 20% *Evernia prunastri* ext.

No relevant *in vitro* studies on *Evernia prunastri* ext. (i.e. OECD TG 442C and OECD TG 442D) were identified in the literature.

Human information

Population studies

Table 2 summarises patch test studies on *Evernia prunastri* ext. involving several thousand dermatitis patients from various countries mainly in Europe. Most of the studies are diagnostic patch test studies.

Table 2. Population studies with *Evernia prunastri* ext.

Method	Results	Remarks/Comments	Reference
Patch test: Retrospective study of 806 selected patients patch tested with <i>Evernia prunastri</i> ext. 1% in petrolatum (pet.).	221/806 (27.4%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2007-2009).	Schnuch et al. (2015).
Patch test: Retrospective study of 1951 selected eczema patients patch tested with <i>Evernia prunastri</i> ext. 2% in pet.	34/1951 (1.7%, 95% CI: 1.1-2.3%) patients were positive.	A retrospective study on patch test data at St John's Institute of Dermatology at St Thomas' Hospital, UK (2011-2012).	Mann et al. (2014).
Patch test: Study of 228 selected patients with occupational dermatitis tested with <i>Evernia prunastri</i> ext. Vehicle and concentration not reported.	2/228 (0.9%) patients were positive.	Study on patch test data from Department of Dermato-Allergology, Copenhagen University Hospital Gentofte, Denmark (2010-2011).	Friis et al. (2013).
Patch test: Retrospective study of 940 selected patients tested with <i>Evernia prunastri</i> ext. in petrolatum. Concentration not reported.	230/940 (24.6%) patients were positive.	A retrospective study on patch test data from Department of Dermatology, University Hospital St Rafaël, Belgium (1990-2011).	Nardelli et al. (2013).
Patch test: Prospective study of 100 selected patients with contact allergy patch tested with <i>Evernia prunastri</i> ext. in petrolatum. Concentration not reported.	25/100 (25%, 95% CI: 16.88-34.66%) patients were positive.	Single-centre, double-blind prospective experimental longitudinal volunteer study at the department of Dermatology of the VU University Medical Centre, The Netherlands (2005-2010).	Nagtegaal et al. (2012).
Patch test: Retrospective study of 1503 consecutive eczema patients	37/1503 (2.5 %) patients were positive.	A retrospective study of patch test data at Department of Dermato-Allergology,	Heisterberg et al. (2011, 2012).

Method	Results	Remarks/Comments	Reference
patch tested with <i>Evernia prunastri</i> ext. 1% in pet.		Copenhagen University Hospital Gentofte, Denmark (2008-2010).	
Patch test: Retrospective study of 157 selected patients (chosen out of 509 patients positive to fragrance allergens) patch tested with <i>Evernia prunastri</i> ext. 2% in pet	Ca. 39/157 (ca. 25%) patients were positive.	A retrospective study of patch test data at the Allergy Clinic of the Department of Dermatology and Venereology, Zagreb University Hospital Center and School of Medicine, Zagreb, Croatia (2001-2005).	Turcic et al. (2011).
Patch test: Retrospective study of 86 selected patients patch tested with <i>Evernia prunastri</i> ext. 2% in pet.	2/86 (2.3%) patients were positive.	A retrospective and descriptive analysis of a patch test study at the Cutaneous Allergy Unit of a tertiary referral hospital, Spain (2004-2008).	Cuesta et al. (2010).
Patch test: Retrospective study of 1213 consecutive patients and 4482 selected patients patch tested with <i>Evernia prunastri</i> ext. 1% in pet.	22/1213 (1.81%, 95% CI: 1.07-2.56%) and 251/4482 (5.59%, 95% CI: 4.9-6.27%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (Information Network of Departments of Dermatology) (2005-2008).	Uter et al. (2010).
Patch test: Prospective study of 320 selected eczema patients patch tested with <i>Evernia prunastri</i> ext. 2% in pet.	6/320 (1.9%) patients were positive.	A prospective analysis of selected eczema patients at the University Medical Center in Groningen, the Netherlands (2005-2007).	van Oosten et al. (2009).
Patch test: Retrospective study of 597 selected patients with a) current allergic dermatitis or b) past allergic dermatitis patch tested with <i>Evernia prunastri</i> ext. 1% in pet.	a) 120/597 (20.1%) and b) 165/597 (27.6%) patients were positive.	A retrospective study of patch test data from patients attending the Department of Cutaneous Allergy at St John's Institute of Dermatology, UK (1982-2007).	White (2009).
Patch test: Prospective study of 15 selected patients with eczematous	0/15 (0%) patients were positive.	A prospective study on patch test data from patients from Italy (2006-2007).	Foti et al. (2008).

Method	Results	Remarks/Comments	Reference
reactions from ketoprofen-containing gels patch tested with <i>Evernia prunastri</i> ext. 2% in pet.			
Patch test: Retrospective study of 2063 consecutive patients patch tested with <i>Evernia prunastri</i> ext. 1% in pet.	46/2063 (2.2%, 95% CI: 1.4-2.6%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (2003-2004).	Schnuch et al. (2007).
Patch test: Retrospective study of a) 28 patients positive to their own shaving product/eau de toilette/perfume and b) 153 negative to their own shaving product/eau de toilette/perfume, patch tested with <i>Evernia prunastri</i> ext. 1% in pet.	a) 8/28 (29%) and b) 14/153 (9%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (1998-2002).	Uter et al. (2007).
Patch test: Study of 30 selected patients with a positive patch test to their own perfumed product patch tested with <i>Evernia prunastri</i> ext. Concentration and vehicle not specified.	9/30 (30%) patients were positive.		Vocanson et al. (2006).
Patch test: Prospective study of 422 selected patients with suspected contact allergy patch tested with <i>Evernia prunastri</i> ext. 2% in pet.	6/422 (1.4%) patients were positive.	A prospective analysis of patients from nine dermatology departments of university hospitals in Korea (2002-2003).	An et al. (2005).
Patch test: Study of 885 consecutive eczema patients patch tested with	28/885 (3.2%) patients were positive or had a follicular patch	Two types of <i>Evernia prunastri</i> ext. (oak moss absolute) were tested, one	Johansen et al., 2002 cited from SCCS (2012).

Method	Results	Remarks/Comments	Reference
<i>Evernia prunastri</i> ext. Concentration and vehicle not reported.	test response.	contaminated by resin acids and one without any detectable resin acids. There was no difference in reactivity between the two types.	
Patch test: Retrospective study of 4900 unselected patients patch tested with <i>Evernia prunastri</i> ext. 1% in pet.	333/4900 (6.8%) patients were positive.	A retrospective study on patch test data from multicentre project IVDK (1996-1999).	Schnuch et al. (2002).
Patch test: Prospective study of 747 selected patients with suspected fragrance allergy patch tested with <i>Evernia prunastri</i> ext. 1% in pet. with 1% sorbitan sesquioleate (SSO).	37/747 (5%) patients were positive.	A prospective analysis of patients from FAZ-Floridsdorf Allergy Centre, Austria (1997-2000).	Wohrl et al. (2001).
Patch test: Study of 226 selected patients sensitive to FM patch tested with <i>Evernia prunastri</i> ext. 1% in pet.	50/226 (22%) patients were positive.	Department of Dermatology, University Hospital, Coimbra, Portugal (1989-1999).	Brites et al. (2000).
Patch test: Retrospective study of 50 patients sensitive to FM patch tested with <i>Evernia prunastri</i> ext. 2% in 1% SSO.	22/50 (44%) patients were positive.	Retrospective study of patch test data. University Hospital Utrecht, The Netherlands (1994-1998).	Hendriks and van Ginkel (1999).
Patch test: Retrospective study of 40 patients sensitive to FM patch tested with <i>Evernia prunastri</i> ext. in pet. Concentration not reported.	12/40 (30%) patients were positive.		Katsarma and Gawkrödger (1999).
Patch test: Study of 167 selected	22/167 (13.2%) patients were	Multicentre study of patch test data from	Larsen et al. (1996).

Method	Results	Remarks/Comments	Reference
patients suspected of fragrance sensitivity patch tested with <i>Evernia prunastri</i> ext. 5% in pet.	positive.	Japan, Northern Ireland, USA, UK, Switzerland and Sweden.	
Patch test: Study of 702 unselected consecutive patients patch tested with <i>Evernia prunastri</i> ext. 1% in pet. with 1% SSO.	18/702 (2.6%) patients were positive.	European multicentre study with 7 different centres.	Frosch et al. (1995a).
Patch test: Study of 702 unselected consecutive patients patch tested with <i>Evernia prunastri</i> ext. 1% in pet.	13/702 (1.9%) patients were positive.	European multicentre study with 7 different centres.	Frosch et al. (1995a).
Patch test: Study of 1072 patients patch tested with <i>Evernia prunastri</i> ext. 1% in pet with 1% SSO.	24/1072 (2.24%) patients were positive.	European multicentre study with 9 different centres.	Frosch et al. (1995b).
Patch test: Retrospective study of 367 selected patients patch tested with <i>Evernia prunastri</i> ext. 1 or 2% in pet. with 5% SSO.	86/367 (23%) patients were positive.	Retrospective study of patch test data from Department of Dermatology, Gentofte Hospital, Denmark (1979-1983 and 1988-1992).	Johansen and Menne (1995).
Patch test: Prospective study of 61 selected patients positive to a fragrance mix patch tested with <i>Evernia prunastri</i> ext. 5% in pet.	21/61 (34%) patients were positive. Control tests in 100 patients not allergic to fragrances showed no positive reactions when tested with <i>Evernia prunastri</i> ext. 5% pet.	Prospective study of patch test data from University of Amsterdam and University of Leiden, The Netherlands (1987).	de Groot et al. (1993).
Patch test: Prospective study of 162 selected patients positive to a fragrance mix	14/162 (8.6%) patients were positive.	Prospective study of patch test data from Dermatologische Klinik und Poliklinik, Germany (1991).	Enders et al. (1989).

Method	Results	Remarks/Comments	Reference
patch tested with <i>Evernia prunastri</i> ext. 1% in pet.			
Patch test: Study of 55 selected patients sensitive to perfumes or after-shave lotions patch with <i>Evernia prunastri</i> ext. 2%. Vehicle not reported.	35/55 (64%) patients were positive.	According to SCCNFP 2000 16 of the 55 patients had a definite history of contact allergy to plants following direct contact.	Thune and Sandberg 1987 cited from SCCNFP (2000).
Patch test: Prospective study of 179 selected patients patch tested <i>Evernia prunastri</i> ext. 10% in pet.	21/179 (11.7%) patients were positive.		de Groot et al. (1985).
Patch test: Study of 20 selected perfume allergic patients patch tested with <i>Evernia prunastri</i> ext. 5% in pet.	3/20 (15%) patients were positive.		Larsen (1977).
Patch test and ROAT ¹ : 30 <i>Evernia prunastri</i> ext. sensitive patients were tested with 0.1% of <i>Evernia prunastri</i> ext. in a ROAT and with a serial dilution of <i>Evernia prunastri</i> ext. 0.00003-2% in a patch test. Vehicle: diethyl phthalate:ethanol 2:98	22/30 patients were positive in the ROAT. Positive reactions were observed in 6/30 (20%) patients at a concentration of 0.0027%.		Andersen et al. (2015).

¹ROAT: Repeated Open Application Test

Table 3 summarises Human Repeat Insult Patch Tests (HRIPTs) with *Evernia prunastri* ext.

Table 3. HRIPT studies with *Evernia prunastri* ext.

Method	Results	Remarks/Comments	Reference
Modified HRIPT (Draize): <i>Evernia prunastri</i> ext. Induction concentration: 5% Vehicle: 1:1 Acetone:Ethanol.	7/53 (13%) tests were positive.		Ehret et al., 1992 cited from SCCNFP (2000).
HRIPT: <i>Evernia prunastri</i> ext. Induction concentration: 5% Vehicle: 3:1 Ethanol:diethyl phthalate (EtOH:DEP).	Sensitisation was observed.		Ford and Api 1990 cited from SCCNFP (2000).
HRIPT: <i>Evernia prunastri</i> ext. Induction concentration: 0.6% Vehicle: 3:1 EtOH:DEP.	0/103 tests were positive.		Ford and Api 1990 cited from SCCNFP (2000).
HRIPT: <i>Evernia prunastri</i> ext. Induction concentration: 1.2% Vehicle: not reported.	0/47 tests were positive.		IFRA 1988 amended in 1992 and 1998 cited from SCCNFP (2000).
HRIPT: <i>Evernia prunastri</i> ext. Induction concentration: 1.2% Vehicle: not reported.	1/48 (2%) tests were positive.		IFRA 1988 amended in 1992 and 1998 cited from SCCNFP (2000).

HRIPT: Human Repeat Insult Patch Test.

Case studies

Table 4 summarises case reports with allergic contact dermatitis where *Evernia prunastri* ext. has been found to be among the causative agents.

Table 4. Case studies with *Evernia prunastri* ext.

Method	Results	Remarks/Comments	Reference
Patch test: A 41-year old female hairdresser with occupational hand dermatitis and scalp dermatitis was patch tested with <i>Evernia prunastri</i> ext.	Positive reaction to <i>Evernia prunastri</i> ext. contained in a perming solution.		Kanerva et al., 1999 cited from SCCS (2012).
Patch test: A woman with ACD ¹ was patch tested with her husband's aftershave lotion and <i>Evernia prunastri</i> ext. 5% in petrolatum.	Positive reaction to <i>Evernia prunastri</i> ext. and her husband's aftershave lotion containing 3% <i>Evernia prunastri</i> ext.		Held et al., 1988 cited from SCCNFP (2000).

¹ACD: Allergic Contact Dermatitis.

A total of 35 results from patch test population studies, 5 HRIPTs and 2 case studies with *Evernia prunastri* ext. are summarised above (Table 2, 3 and 4). As shown in Table 2 the positive patch test frequencies from all of the reported patch test population studies vary between 0 and 64% in dermatitis patients. For unselected/consecutive dermatitis patients, positive reactions range between 1.8 and 6.8% (7 studies) and for selected dermatitis patients positive reactions range between 0 and 64% with 21/28 studies reporting frequencies equal to or higher than 5%. The total number of published cases is > 1900. Sensitisation was reported in 3/5 HRIPT studies with no reactions at 0.6%, 0-2% at 1.2% and 13% at 5% induction concentrations of *Evernia prunastri* ext. The Research Institute for Fragrance Materials, Inc. (RIFM) has on the basis of these and other unpublished studies deducted a NOEL²⁰-HRIPT (induction) of 700 µg/cm², a NOEL-HMT of 1724 µg/cm² and a LOEL-HRIPT/HMT²¹ (induction) of 1417 µg/cm². In addition, based on weight of evidence, a No Expected Sensitization Induction Level (NESIL) of 700 µg/cm² was established for *Evernia prunastri* ext. by the RIFM Expert Panel (IFRA, 2008e).

According to SCCS (2012) *Evernia prunastri* ext. is used in volumes less than 175 ton per year in perfume formulations. It has been reported that in consumer products containing fragrance allergens that are required to be labelled 4.6% of 88 tested deodorants (in 2007), 0.8% of a total of 516 consumer products; 4% of a total of 300 consumer products; ca. 1% of 3000 products and 0% of children's cosmetics were labelled to contain *Evernia prunastri* ext. (Wijnhoven et al., 2008; Buckley, 2007; Schnuch et al., 2009 and Poulsen & Schmidt, 2007 cited from SCCS (2012)). Based on these data SCCS (2012) concluded that *Evernia prunastri* ext. was among the least used fragrance ingredients in cosmetics and other consumer products.

The IFRA standard limits for *Evernia prunastri* ext. in different IFRA QRA product categories reported by IFRA (2008e and 2015) are shown in table 5.

²⁰ NOEL: No Observed Effect Level.

²¹ LOEL-HRIPT/HMT: Lowest Observed Effect Level-Human Repeat Insult Patch Test/Human Maximisation test.

Table 5. The IFRA standard limits for *Evernia prunastri* ext. in IFRA QRA product categories.

IFRA category	QRA product	Product type that drives the category consumer exposure level	IFRA standard limits
Category 1		Lip products	0.02%
Category 2		Deodorants/antiperspirants	0.03%
Category 3		Hydroalcohols for shaved skin	0.1%
Category 4		Hydroalcohols for unshaved skin	0.1%
Category 5		Hand cream	0.1%
Category 6		Mouthwash	0.5%
Category 7		Intimate wipes	0.1%
Category 8		Hair styling aids	0.1%
Category 9		Rinse-off hair conditioners	0.1%
Category 10		Hard surface cleaners	0.1%
Category 11		Candles	Not restricted

IFRA: International Fragrance Association, QRA: Quantitative Risk Assessment.

Evernia prunastri ext. is not registered under the REACH regulation.

Summary and discussion of skin sensitization

Human data

A total of 35 results from patch test population studies, 5 HRIPTs and 2 case studies were identified with *Evernia prunastri* ext. The positive patch test frequencies from all of the reported patch test population studies vary between 0 and 64% in dermatitis patients. In studies with unselected/consecutive dermatitis patients positive reactions range between 1.8 and 6.8% (7 studies) and in studies with selected dermatitis patients positive reactions range between 0 and 64% (28 studies). The total number of published cases is > 1900. A LOEL-HRIPT/HMT (induction) of 1417 µg/cm² was established for *Evernia prunastri* ext. by the RIFM Expert Panel based on unpublished reports.

Non-human data

A total of 1 LLNA, 1 LLNA *ex vivo* BrdU and 1 GPMT were identified testing skin sensitising effects of *Evernia prunastri* ext. The reported EC3 value for *Evernia prunastri* ext. was 3.9% in the LLNA and 3.4% in the LLNA *ex vivo* BrdU. In the GPMT sensitisation was observed in 63% of the animals after an intradermal induction dose of 20% *Evernia prunastri* ext.

No relevant *in vitro* studies on *Evernia prunastri* ext. (i.e. OECD TG 442C and OECD TG 442D) were identified in the literature.

Exposure

According to data from IFRA (2008e) the exposure of *Evernia prunastri* ext. when used as fragrance in cosmetics and in other consumer products appears to be low.

Comparison with criteria

For unselected/consecutive dermatitis patients positive reactions range between 1.81 and 6.8% i.e. all studies are reporting frequencies higher than 1%. For selected dermatitis patients positive reactions range between 0 and 64% with 23 out of 28 studies reporting frequencies higher than 2%.

In addition to this there are more than 1900 published cases of positive patch test reactions to *Evernia prunastri* ext. According to the CLP criteria a frequency $\geq 1\%$ for unselected/consecutive dermatitis patients and/or $\geq 2\%$ for selected dermatitis patients and/or a total number of published cases ≥ 100 , equals a high frequency of occurrence of skin sensitisation (Table 3.4.2-b) (ECHA, 2015). The collected data described above from patch test studies show that *Evernia prunastri* ext. causes a *high frequency* of occurrence of skin sensitisation based on these three types of information.

In regard to the HRIPT/HMT data the positive response reported at $> 500 \mu\text{g}/\text{cm}^2$ for HRIPT/HMT induction threshold indicate evidence for sub-category 1B according to Annex I: 3.4.2.2.2.2.

In the 2 LLNAs EC3 values were 3.4 and 3.9%. According to the CLP Regulation an EC3 value $\leq 2\%$ indicates classification of a substance in sub-category 1A whereas an EC3 value $> 2\%$ indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.). Thus, both studies indicate classification of *Evernia prunastri* ext. in sub-category 1B.

In the GPMT sensitisation was observed in 63% of the animals after an intradermal induction dose of 20%. According to the CLP criteria a positive response $\geq 30\%$ of the animals responding at $> 1\%$ intradermal induction dose indicates classification of a substance in sub-category 1B (Annex I: 3.4.2.2.3.2.) and thus, this study indicates classification of *Evernia prunastri* ext. into sub-category 1B.

Overall, there is clear evidence for classification in sub-category 1A based on the human patch test studies showing a high frequency of occurrence of skin sensitisation and the total number of cases combined with the estimated low exposure. The results from the animal studies indicate classification in sub-category 1B. A classification as a skin sensitiser in sub-category 1A is thus warranted for *Evernia prunastri* ext.

Conclusions on classification and labelling

Based on the high frequency of sensitisation observed in human patch test studies and the high number of published cases combined with the estimated low exposure, a classification of *Evernia prunastri* ext. as a skin sensitiser in sub-category 1A is justified.

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Evaluation of selected sensitising fragrance substances

Fragrances are widely used in many different types of consumer products and consumers may thus be exposed to fragrances from many different sources. The exposure can be substantial despite the fact that fragrances are typically used in relatively low concentrations in individual products. Skin sensitisation (contact allergy) is a critical effect for human health for many fragrances. The purpose of this project was to retrieve and review the available data for 42 selected sensitising fragrance substances in order to assess whether these substances are potent enough fulfil the criteria for classification as strong sensitizers in sub-category 1A according to the CLP Regulation on classification, labelling and packaging. A classification as a strong sensitizer in category 1A will impact the level of information that has to be supplied on the label on e.g. washing and cleaning products containing such substances. The more stringent labelling requirements for products containing potent sensitizers will provide better protection of users (both consumers and workers) as it will allow sensitised individuals to take precautionary measures to prevent direct skin contact with such products.

Parfume er vidt udbredt i mange forskellige typer forbrugerprodukter, og forbrugere bliver eksponeret for parfume fra mange forskellige kilder. Eksponeringen kan være betragtelig på trods af, at parfumestoffer generelt anvendes i relativt lave koncentrationer i de enkelte produkter. Hudsensibilisering (kontakt allergi) er en af de kritiske effekter for sundheden for mange parfumestoffer. Formålet med dette projekt var at søge og vurdere de tilgængelige data for 42 udvalgte sensibiliserende parfumestoffer for at vurdere, om disse stoffer er potente nok til at opfylde kriterierne for klassificering som stærkt sensibiliserende stoffer i kategori 1A i henhold til CLP forordningen om klassificering, mærkning og emballering. En klassificering som stærkt sensibiliserende i kategori 1A vil have betydning for mærkningen af f.eks. vaske- og rengøringsmidler indeholdende sådanne stoffer. De strengere mærkningskrav for produkter indeholdende potente allergener vil give en bedre beskyttelse af brugerne (både forbrugere og arbejdstagere), da det vil give sensibiliserede personer den nødvendige information i forhold til at forebygge direkte hudkontakt med sådanne produkter.



CONCLUSION ON PESTICIDE PEER REVIEW

Conclusion on the peer review of the pesticide risk assessment of the active substance plant oils/spearmint oil¹

European Food Safety Authority²

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SUMMARY

Spearmint oil is one of the 295 substances of the fourth stage of the review programme covered by Commission Regulation (EC) No 2229/2004³, as amended by Commission Regulation (EC) No 1095/2007⁴.

Spearmint oil was included in Annex I to Directive 91/414/EEC on 18 December 2008 pursuant to Article 24b of the Regulation (EC) No 2229/2004 (hereinafter referred to as „the Regulation’) and has subsequently been deemed to be approved under Regulation (EC) No 1107/2009⁵, in accordance with Commission Implementing Regulation (EU) No 540/2011⁶, as amended by Commission Implementing Regulation (EU) No 541/2011⁷. In accordance with Article 25a of the Regulation, as amended by Commission Regulation (EU) No 114/2010⁸, the European Food Safety Authority (EFSA) is required to deliver by 31 December 2012 its view on the draft review report submitted by the European Commission in accordance with Article 25(1) of the Regulation. This review report was established as a result of the initial evaluation provided by the designated rapporteur Member State in the Draft Assessment Report (DAR). The EFSA therefore organised a peer review of the DAR. The conclusions of the peer review are set out in this report.

Sweden being the designated rapporteur Member State submitted the DAR on spearmint oil in accordance with the provisions of Article 22(1) of the Regulation, which was received by the EFSA on 9 November 2007. The peer review was initiated on 18 June 2008 by dispatching the DAR for consultation of the notifier (XEDA International). Subsequently the DAR was dispatched for consultation of the Member States on 24 February 2011. Following consideration of the comments received on the DAR, it was concluded that EFSA should conduct a focused peer review in the areas of mammalian toxicology and deliver its conclusions on spearmint oil.

The conclusions laid down in this report were reached on the basis of the evaluation of the representative uses of spearmint oil as a plant growth regulator on potatoes as proposed by the notifier. Full details of the representative uses can be found in Appendix A to this report.

¹ On request from the European Commission, Question No EFSA-Q-2009-00293 adopted on 16 December 2011

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³ OJ L 379, 24.12.2004, p.13

⁴ OJ L 246, 21.9.2007, p.19

⁵ OJ L 309, 24.11.2009, p.1

⁶ OJ L 153, 11.6.2011, p.1

⁷ OJ L 153, 11.6.2011, p.187

⁸ OJ L 37, 10.2.2010, p.12

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In the area of identity, physical/chemical/technical properties and methods of analysis a data gap was identified for an Annex II data package for the active components that make up spearmint oil. Data gaps for the formulation are auto-flammability, accelerated storage and shelf life. No methods of analysis were available for products of plant and animal origin and for soil, water and air.

In the mammalian toxicology area data gaps were identified for the definition of the toxicological profile of spearmint oil; the database was insufficient to set reference values and the risk assessment is inconclusive.

In the residues section it was concluded that significant levels of residues of (R)-carvone will occur in potatoes upon application of spearmint oil according to the proposed use scenario. However, several data gaps were identified and a final residue definition for risk assessment and monitoring could not be derived. Furthermore, in absence of agreed toxicological reference values a consumer risk assessment could not be performed.

The environmental fate and behaviour section of the dossier was empty. Consequently 4 data gaps have been identified. Quantitative structure activity relationship (QSAR) approaches have been used to obtain more uncertain than usual input parameters to carry out groundwater exposure modelling for the main constituent of spearmint oil. When the uncertainty in the use of the QSAR approach is included in the modelling strategy, these modelling results indicate a high potential for groundwater contamination by the pesticide active substance carvone, consequent to the representative use assessed, in the situation when treated potatoes are used as seed potatoes.

The data set for the ecotoxicological assessments was not sufficient, therefore the risk assessments for non-target organisms could not be finalised.

KEY WORDS

Spearmint oil, (R)-carvone, L-carvone, (5R)-2-methyl-5-(prop-1-ene-2-yl)cyclohex-2-en-1-one, peer review, risk assessment, pesticide, plant growth regulator

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BACKGROUND

Spearmint oil is one of the 295 substances of the fourth stage of the review programme covered by Commission Regulation (EC) No 2229/2004⁹, as amended by Commission Regulation (EC) No 1095/2007¹⁰.

Spearmint oil was included in Annex I to Directive 91/414/EEC on 18 December 2008 pursuant to Article 24b of the Regulation (EC) No 2229/2004 (hereinafter referred to as 'the Regulation') and has subsequently been deemed to be approved under Regulation (EC) No 1107/2009¹¹, in accordance with Commission Implementing Regulation (EU) No 540/2011¹², as amended by Commission Implementing Regulation (EU) No 541/2011¹³. In accordance with Article 25a of the Regulation, as amended by Commission Regulation (EU) No 114/2010¹⁴ the European Food Safety Authority (EFSA) is required to deliver by 31 December 2012 its view on the draft review report submitted by the European Commission in accordance with Article 25(1) of the Regulation (European Commission, 2008). This review report was established as a result of the initial evaluation provided by the designated rapporteur Member State in the Draft Assessment Report (DAR). The EFSA therefore organised a peer review of the DAR. The conclusions of the peer review are set out in this report.

Sweden being the designated rapporteur Member State submitted the DAR on spearmint oil in accordance with the provisions of Article 22(1) of the Regulation, which was received by the EFSA on 9 November 2007 (Sweden, 2007). The peer review was initiated on 18 June 2008 by dispatching the DAR for consultation of the notifier (XEDA International). Subsequently the DAR was dispatched for consultation of the Member States on 24 February 2011. In addition, the EFSA conducted a public consultation on the DAR. The comments received were collated by the EFSA and forwarded to the RMS for compilation and evaluation in the format of a Reporting Table. The notifier was invited to respond to the comments in column 3 of the Reporting Table. The comments were evaluated by the RMS in column 3 of the Reporting Table.

The scope of the peer review was considered in a telephone conference between the EFSA, the RMS, and the European Commission on 20 June 2011. On the basis of the comments received and the RMS' evaluation thereof it was concluded that the EFSA should organise a consultation with Member State experts in the areas of mammalian toxicology.

The outcome of the telephone conference, together with EFSA's further consideration of the comments is reflected in the conclusions set out in column 4 of the Reporting Table. All points that were identified as unresolved at the end of the comment evaluation phase and which required further consideration, including those issues to be considered in consultation with Member State experts, and additional information to be submitted by the notifier, were compiled by the EFSA in the format of an Evaluation Table.

The conclusions arising from the consideration by the EFSA, and as appropriate by the RMS, of the points identified in the Evaluation Table, together with the outcome of the expert discussions where these took place, were reported in the final column of the Evaluation Table.

A final consultation on the conclusions arising from the peer review of the risk assessment took place with Member States via a written procedure in November/December 2011.

⁹ OJ L 379, 24.12.2004, p.13

¹⁰ OJ L 246, 21.9.2007, p.19

¹¹ OJ L 309, 24.11.2009, p.1

¹² OJ L 153, 11.6.2011, p.1

¹³ OJ L 153, 11.6.2011, p.187

¹⁴ OJ L 37, 10.2.2010, p.12

This conclusion report summarises the outcome of the peer review of the risk assessment on the active substance and the representative formulation evaluated on the basis of the representative uses as a plant growth regulator on potatoes, as proposed by the notifier. A list of the relevant end points for the active substance as well as the formulation is provided in Appendix A. In addition, a key supporting document to this conclusion is the Peer Review Report, which is a compilation of the documentation developed to evaluate and address all issues raised in the peer review, from the initial commenting phase to the conclusion. The Peer Review Report (EFSA, 2011) comprises the following documents, in which all views expressed during the course of the peer review, including minority views, can be found:

- the comments received on the DAR,
- the Reporting Table (20 June 2011)
- the Evaluation Table (12 December 2011)
- the report(s) of the scientific consultation with Member State experts (where relevant),
- the comments received on the assessment of the points of clarification (where relevant),
- the comments received on the draft EFSA conclusion.

Given the importance of the DAR including its addendum (compiled version of November 2011 containing all individually submitted addenda (Sweden, 2011)) and the Peer Review Report, both documents are considered respectively as background documents A and B to this conclusion.

THE ACTIVE SUBSTANCE AND THE FORMULATED PRODUCT

Spearmint oil is the given name for a mixture that contains (R)-carvone as the main constituent and other identified compounds. There is no ISO common name. A number of the constituents of spearmint oil have one or more asymmetric carbon atoms, so enantiomers and diastereoisomers are present in the mixture as applied. A number of the risk characterisations presented in this conclusion do not address the potential for change in enantiomer ratios following use.

The representative formulated product for the evaluation was „BIOX-M’ a hot fogging concentrate containing 949 g/l spearmint oil.

The representative uses evaluated is as a post harvest treatment to potatoes as a sprout suppressant. Full details of the GAP can be found in the list of end points in Appendix A.

CONCLUSIONS OF THE EVALUATION

1. Identity, physical/chemical/technical properties and methods of analysis

The notifier has stated that all of the compounds in spearmint oil are the active substances and full details of their identity and content range are given in Appendix C and the Addendum to Volume 4 of the DAR (Sweden, 2011). It should be noted that the ratio of isomers was not confirmed by the analytical method used. There are no relevant impurities. There is no FAO specification.

No information was given on the level of microbial contamination and the mechanism for the control of such contamination and its possible increase on storage.

A full Annex II data package was identified as a data gap for the active components.

The following data gaps were identified for the formulated product: auto-flammability, accelerated storage and shelf life.

Data gaps have been identified for methods of analysis for products of plant and animal origin and soil, water and air. A method for body fluids and tissues is not required as there is no classification for toxic or very toxic.

2. Mammalian toxicity

Spearmint oil was discussed in the Pesticides Peer Review meeting 88 (September 2011).

For spearmint oil the only original toxicity studies available in the dossier are acute oral, dermal, inhalation, irritation and skin sensitisation studies, showing that spearmint oil is not acutely toxic, not irritating to skin and eye, but it is a skin sensitiser (R43 proposed). Spearmint oil is composed of alicyclic hydrocarbons, and meets the physico-chemical characteristics for assignment of the risk phrase R65 “Harmful: may cause lung damage if swallowed”.

The amount of (R)-carvone in spearmint oil was stated in volume 4 as being up to 75-80%. The question was raised whether the studies performed on carvone should be considered at all for spearmint oil as at least 20% of the technical material is missing. Furthermore the raw data were not available. From the open literature, the carvone tested in many of the studies could not be well identified.

The experts concluded that the majority of the data is based on carvone and reviews from other authorities that did not allow for an independent assessment. Data gaps were identified for toxicological information on spearmint oil, including genotoxicity studies. The database is therefore insufficient to set reference values for spearmint oil, or for (R)- or (S)-carvone, and the risk assessment is inconclusive.

3. Residues

The assessment in the residue section below is based on the guidance documents listed in the document 1607/VI/97 rev.2 (European Commission, 1999), and the JMPR recommendations on livestock burden calculations stated in the 2004 and 2007 JMPR reports.

No metabolism study with spearmint oil was submitted. It was argued that the high volatility of spearmint oil contraindicated significant absorption and residues were unlikely to occur. The main component of spearmint oil is (R)-carvone (up to 75-80%).

The formation of residues of (R)-carvone and (S)-carvone upon treatment of potatoes has previously been assessed in the DAR for carvone (The Netherlands, 2000). Only information for (R)-carvone is considered here. Within the tested three weeks of storage residue levels increased in both potato peel and tubers, indicating the compound was gradually absorbed from the air into the crop. (R)-carvone was a major residue at any sampling time investigated. Also residues of metabolite neodihydrocarveol, a diastereoisomer of dihydrocarveol, were found to be significant (14% and 62% of the identified residue in tubers and peeled tubers, respectively, after 3 weeks). Identification of the terminal residue might not be complete in this study. However, it can be reasonably deduced that significant residues are likely to occur and that investigation of the nature of residues of spearmint oil cannot be waived, taking also into account the presence of compounds in spearmint oil other than (R)-carvone. In addition, residue trials with spearmint oil performed according to the proposed use scenario demonstrated that significant levels of (R)-carvone were present in potatoes. Other compounds were not analysed for in these trials.

Subsequently, a data gap had to be set for data addressing the nature of residues in potatoes. Currently, a residue definition for consumer risk assessment can not be concluded. Furthermore, the open issue regarding clarification of the toxicological role of compounds present in spearmint oil other than (R)-carvone (refer to section 2) may possibly also impact the selection of an appropriate residue definition for risk assessment. Accordingly, it is deemed premature to propose a residue definition for monitoring even if based on the available data (R)-carvone might appear a suitable marker.

In addition, a data gap was set for a sufficient number of residue trials in potatoes according to critical GAP criteria, and for a freezer storage stability study to validate the results found in the residue studies. Possibly, data on processed potatoes may become necessary to demonstrate reduction of residue levels. Lastly, data and information to address potential residues in food of animal origin are required since treated potatoes or their products may be fed to livestock and the trigger value for livestock dietary exposure is exceeded.

Although the available data would permit a preliminary assessment of consumer intakes of (R)-carvone from the consumption of potatoes, a reliable consumer risk assessment cannot be performed due to several data gaps that could have an impact on the final result and, not least, due to the absence of agreed toxicological reference values (refer to section 2).

4. Environmental fate and behaviour

Measured experimental data for any of the components that constitute the active substance spearmint oil and how they behave in environmental matrices was not available in the applicants dossier. For the predominant component reported to be (R)-carvone, quantitative structure activity relationship calculations (QSAR) estimates were provided for the properties water solubility (WSKOW v.1.41: 367.1 mg/L at 25°C), vapour pressure (MPBPWIN v.1.42: 17.3 Pa at 25°C), soil DT₅₀ (30 days) water and sediment DT₅₀, (15 and 135 days respectively) (DT values calculated with Biowin v4.10 with ultimate values transferred to the Level III fugacity model LEVEL3NT.EXE), soil adsorption (PCKOC v1.66: K_{doc} of 124 mL/g) and for indirect photolytic reaction with hydroxyl radicals in the upper atmosphere (AOP v.1.92; atmospheric half life 0.114 days), all models contained in EPI Suite v3.20¹⁵. For an other active component (listed in row 2 in Table C.1.2-1 of the Addendum to Volume 4 of the DAR; Sweden, 2011)¹⁶ these values were: water solubility (WSKOW v.1.42: 4.6 mg/L at 25°C), vapour pressure (MPBPVP v.1.43: 193 Pa at 25°C), soil DT₅₀ (30 days) water and sediment DT₅₀, (15 and 135 days respectively) (DT values calculated with Biowin v4.10 with ultimate values transferred to the Level III fugacity model LEVEL3NT.EXE), soil adsorption (KOCWIN v2.0: K_{doc} of 1120 mL/g) and for indirect photolytic reaction with hydroxyl radicals in the upper atmosphere (AOP v.1.92; atmospheric half life 0.074 days) all models contained in EPI Suite v4.1¹⁷.

In line with the assessment of other substances, as QSAR estimates have more uncertainty than experimentally measured values, it was agreed that predicted environmental concentration (PEC) estimates could be made using the following substance values: soil DT₅₀ 300 days, water DT₅₀ 150 days, sediment DT₅₀ 1000 days for (R)-carvone and for the component listed in row 2 in Table C.1.2-1 of the Addendum to Volume 4 of the DAR (Sweden, 2011), with K_{doc} being 12.4mL/g and 112mL/g for each of these compounds respectively (all these values are a factor of 10 more conservative than the their QSAR estimates. This reflects the reported uncertainty in these key QSAR estimations). In addition, for groundwater PEC, the actual QSAR values for vapour pressure and water solubility were used as modelling input. The PEC in soil, surface water and sediment (calculated using the FOCUS (2001) step 2 approach (version 1.1 of the steps 1-2 in FOCUS calculator) for surface water and sediment) and groundwater (calculated using FOCUS (FOCUS, 2009) scenarios and the model PEARL 4.4.4¹⁸) can be found in appendix A. The basis for these calculations was carvone residues measure in stored potatoes at the time potatoes are removed from storage (as described in section 3), to cover the situation when seed potatoes are treated. Using these PEC groundwater calculations all 9 pertinent FOCUS vulnerable shallow groundwater scenarios were predicted to have carvone present in groundwater at concentrations above the parametric drinking water limit for pesticides¹⁹ of 0.1µg/L. Consequently a data gap is identified to provide a more refined groundwater exposure assessment for carvone (see section 7). The provision of less uncertain (R)-carvone soil DT₅₀ and adsorption values than those provided by QSAR estimates would be one way of addressing this data gap. Because of its predicted higher soil adsorption and higher vapour pressure, any refined environmental exposure assessment for carvone would cover the exposure that would occur for the component listed in row 2 in Table C.1.2-1 of the Addendum to Volume 4 of the DAR (Sweden, 2011) in soil, natural surface water systems and groundwater. However until a refined exposure assessment becomes available for carvone, the environmental exposure assessment for the compound listed in row 2 in Table C.1.2-1 of the Addendum to Volume 4 of the DAR (Sweden, 2011) for these compartments also has to be considered a data gap (see section 7). Data gaps are also identified for the environmental exposure assessment to be addressed for the majority of the other components that

¹⁵ Copyright U.S. Environmental Protection Agency.

¹⁶ The components of the active substance spearmint oil other than (R)-carvone are not disclosed in the published EFSA conclusion upon advice from the European Commission following a confidentiality claim made by the applicant as provided for in Article 14 of Council Directive 91/414/EEC.

¹⁷ Copyright U.S. Environmental Protection Agency.

¹⁸ Simulations utilised the agreed Q10 of 2.58 (following EFSA, 2007) and Walker equation coefficient of 0.7

¹⁹ The pertinent legislation Council Directive 98/83/EC (OJ L330, 5.12.1998, p.32) defines plant growth regulators as pesticides. Carvone is approved for use as a plant growth regulator.

make up spearmint oil as no assessment was available for them (see section 7). A data gap is also identified for information on the potential transformation products in soil of any of the constituents that are reported to make up the active substance, that it cannot be excluded would be residues on treated potatoes when they are used as seed potatoes (see section 7). Though (R)-carvone and the component listed in row 2 in Table C.1.2-1 of the Addendum to Volume 4 of the DAR (Sweden, 2011) are volatile, they are not expected to be subject to long range atmospheric transport as their atmospheric half lives, consequent to photooxidative reaction with hydroxyl radicals present in the upper atmosphere are calculated to be less than 2 days. Assessments to address the potential for long range transport for the majority of the other components that make up spearmint oil have been identified as a data gap (see section 7).

In the Member States' and EFSA's comments on the DAR it was discussed that sewage water including cleaning water (that could involve high volumes of water used to wash potatoes when removed from store) would be collected and transported to a management facility for chemical waste. In this situation it was appropriate to assume that environmental exposure from these activities would be precluded (negligible). Other approaches, to adequately manage and appropriately dispose of any water used to wash potatoes and handling equipment such as grading conveyors and packaging equipment might also be devised by Member States. This issue has therefore been included in section 8 of this conclusion, to make it clear that such measures, or local risk assessments, consequent to the disposal of wash waters will be needed, as the outcome from a range of the possible practices has not been addressed in this EU level assessment. Neither the applicant nor the RMS provided environmental exposure assessments where volatile spearmint oil components might be re-deposited on soil or natural surface water systems when potato stores are vented prior to removal of potatoes from store for sale to consumers. When asked to provide such an assessment, the argument was made and accepted that environmental exposure from this situation was likely to be lower than when treated potatoes were used as seed potatoes. Consequently, as the environmental exposure and risk assessment from the use on seed potatoes remains open, these assessments for potatoes destined for human consumption also remain open.

5. Ecotoxicology

No experimental data were available for the ecotoxicological risk assessments for spearmint oil with the exception of the available mammalian data from section 2.

Exposure of the terrestrial environment cannot be excluded if residues (constituents of spearmint oil and their transformation products) remain on potato tubers when they are planted out. The aquatic environment can be contaminated via soil. Moreover contamination of soils and surface waters via aerial deposition of the potentially volatile constituents of spearmint oil cannot be excluded as well (see section 4). Therefore non-target organisms can potentially be exposed to these residues, directly or indirectly via the food chain.

Available data on mammals indicated potential low acute toxicity for spearmint oil (see section 2). The treated tubers are planted in soil therefore the availability of them to birds and to some wild mammal species was considered to be limited. Moreover potato plants (leaves) are not palatable for birds. Nevertheless, since other essential information (e.g. long-term toxicity, systemic properties or potential for bioaccumulation of the constituents) is unknown, complete and reliable assessments for birds and mammals could not be performed.

With regard to the aquatic organisms, acute toxicity of the single compound of spearmint oil, (R)-carvone, was estimated by QSAR analysis for fish and daphnids and the toxicity to algae was also estimated. If these data were compared with the estimated PEC_{sw} values, low risk to aquatic organisms could be concluded for (R)-carvone. Non peer-reviewed data indicated however that another constituent of spearmint oil (listed in row 2 in Table C.1.2-1 of the Addendum to Volume 4 of

the DAR; Sweden, 2011) was more toxic to aquatic organisms than the estimated values for (R)-carvone. No assessments were available for long-term scale, neither any reliable assessments were available for the other constituents of spearmint oil.

As for birds, mammals and aquatic organisms, neither the magnitude of the environmental contamination nor the toxicological profile of the constituents were well characterised for bees, non-target arthropods, earthworms, soil macro and micro-organisms, terrestrial non-target plants and regarding the potential effects on biological methods of sewage treatment. Therefore a data gap was identified for the necessary data and assessments for non-target terrestrial and aquatic organisms.

6. Overview of the risk assessment of compounds listed in residue definitions triggering assessment of effects data for the environmental compartments

6.1. Soil

Compound (name and/or code)	Persistence	Ecotoxicology
carvone (expected to be (R)-carvone / L-carvone / (5R)-2-methyl-5-(prop-1-ene-2-yl)cyclohex-2-en-1-one on the basis of taste and smell)	moderately persistent based on QSAR DT ₅₀ of 30days,	Data gap
[REDACTED]	moderately persistent based on QSAR DT ₅₀ of 30days,	Data gap
[REDACTED]	Data gap	Data gap
[REDACTED]	Data gap	Data gap
[REDACTED]	Data gap	Data gap
[REDACTED]	Data gap	Data gap
Other less prominent constituents, see Addendum to Volume 4 of the DAR for further details.	Data gap	Data gap

6.2. Ground water

Compound (name and/or code)	Mobility in soil	>0.1 µg/L 1m depth for the representative uses (at least one FOCUS scenario or relevant lysimeter)	Pesticidal activity	Toxicological relevance	Ecotoxicological activity
carvone (expected to (R)- carvone)	High mobility QSAR estimated K_{doc} 124mL/g	Yes at all 9 FOCUS scenarios with the available uncertain estimates.	Yes	No data available	Data gap
[REDACTED]	Low mobility QSAR estimated K_{doc} 1120mL/g	Data gap	Yes, claimed	No data available	Data gap
[REDACTED]	Data gap	-	Yes, claimed	No data available	Data gap
[REDACTED]	Data gap	-	Yes, claimed	No data available	Data gap
[REDACTED]	Data gap	-	Yes, claimed	No data available	Data gap

Compound (name and/or code)	Mobility in soil	>0.1 µg/L 1m depth for the representative uses (at least one FOCUS scenario or relevant lysimeter)	Pesticidal activity	Toxicological relevance	Ecotoxicological activity
[REDACTED] [REDACTED] [REDACTED] [REDACTED]	Data gap	-	Yes, claimed	No data available	Data gap
Other less prominent constituents, see Addendum to Volume 4 of the DAR for further details	Data gap	-	Yes, claimed	No data available	Data gap

6.3. Surface water and sediment

Compound (name and/or code)	Ecotoxicology
carvone (expected to (R)-carvone)	Data gap
[REDACTED] [REDACTED]	Data gap
[REDACTED] [REDACTED] [REDACTED]	Data gap
[REDACTED] [REDACTED]	Data gap

[REDACTED] [REDACTED]	Data gap
[REDACTED] [REDACTED]	Data gap
Other less prominent constituents, see Addendum to Volume 4 of the DAR for further details	Data gap

6.4. Air

Compound (name and/or code)	Toxicology
carvone (expected to (R)-carvone)	No data available
[REDACTED] [REDACTED]	No data available
[REDACTED] [REDACTED] [REDACTED]	No data available
[REDACTED] [REDACTED]	No data available
[REDACTED] [REDACTED]	No data available
[REDACTED] [REDACTED]	No data available
Other less prominent constituents, see Addendum to Volume 4 of the DAR for further details	No data available

7. List of studies to be generated, still ongoing or available but not peer reviewed

This is a complete list of the data gaps identified during the peer review process, including those areas where a study may have been made available during the peer review process but not considered for procedural reasons (without prejudice to the provisions of Article 7 of Directive 91/414/EEC concerning information on potentially harmful effects).

- Annex II data package for the active components (relevant for all representative uses evaluated; submission date proposed by the notifier: unknown; see section 1)
- For the formulation auto-flammability, accelerated storage and shelf life (relevant for all representative uses evaluated; submission date proposed by the notifier: unknown; see section 1)
- Methods of analysis for products of plant and animal origin, soil, water and air (relevant for all representative uses evaluated ; submission date proposed by the notifier: unknown; see section 1)
- Toxicological profile of spearmint oil except for acute toxicity (relevant for all representative uses evaluated; submission date proposed by the notifier: unknown; see section 2)
- A freezer storage stability study (relevant for all representative uses evaluated; submission date proposed by the notifier: new study available but not eligible for peer review; see section 3)
- Data on the nature of residues of spearmint oil in potato tuber taking into account the critical use scenario (relevant for all representative uses evaluated; submission date proposed by the notifier: unknown; see section 3)
- Data and information to address residues in livestock or justification of the potential non-relevance of such data (relevant for all representative uses evaluated; submission date proposed by the notifier: unknown; see section 3)
- A sufficient number of critical GAP conforming residue trials with spearmint oil in potatoes, and possibly residue data on processed potatoes to sufficiently demonstrate reduction of residues (relevant for all representative uses evaluated; submission date proposed by the notifier: unknown; see section 3)
- Information to allow a more refined groundwater exposure assessment for the active substance constituents (R)-carvone and the component listed in row 2 in Table C.1.2-1 of the Addendum to Volume 4 of the DAR (Sweden, 2011) are considered necessary (relevant for all representative uses evaluated; submission date proposed by the notifier: unknown; see section 4)
- A satisfactory exposure assessment for soil, surface water and sediment for the active substance constituent listed in row 2 in Table C.1.2-1 of the Addendum to Volume 4 of the DAR (Sweden, 2011) was not available (relevant for all representative uses evaluated; submission date proposed by the notifier: unknown; see section 4)
- Environmental exposure assessments (including potential for long range atmospheric transport) were not available for the majority of the constituents that are reported to make up the active substance (relevant for all representative uses evaluated; submission date proposed by the notifier: unknown; see section 4)
- Information on the potential transformation products in soil of any of the constituents that are reported to make up the active substance, that it cannot be excluded would still be present on seed potatoes when planted out, was not available (relevant for all representative uses evaluated; submission date proposed by the notifier: unknown; see section 4)

- Ecotoxicological risk assessments for non-target terrestrial and aquatic organisms (relevant for the representative use in potato; submission date proposed by the notifier: unknown; see section 5)

8. Particular conditions proposed to be taken into account to manage the risk(s) identified

- Management measures tailored to local practice and legislation need to be put in place to control the waste disposal of potato wash water or wash water from the cleaning of potato grading or packaging equipment, to limit spearmint oil constituents entering sewers or surface water drains to negate possible impacts on natural water systems; see section 4).

9. Concerns

9.1. Issues that could not be finalised

An issue is listed as an issue that could not be finalised where there is not enough information available to perform an assessment, even at the lowest tier level, for the representative uses in line with the Uniform Principles of Annex VI to Directive 91/414/EEC and where the issue is of such importance that it could, when finalised, become a concern (which would also be listed as a critical area of concern if it is of relevance to all representative uses).

1. Environmental exposure assessments were not available for the majority of the constituents that are reported to make up the active substance.
2. The soil, surface water, sediment and groundwater exposure assessments for the active substance constituent listed in row 2 in Table C.1.2-1 of the Addendum to Volume 4 of the DAR (Sweden, 2011) could not be finalised with the available information.
3. Soil, natural surface water system (via drainage) and groundwater exposure assessments for the potential transformation products in soil of any of the constituents that are reported to make up the active substance, that might be present on seed potatoes when planted out could not be finalised.
4. The data set for the ecotoxicological assessments was insufficient, therefore the risk characterization (risk assessments) for non-target organisms could not be finalised.

9.2. Critical areas of concern

An issue is listed as a critical area of concern where there is enough information available to perform an assessment for the representative uses in line with the Uniform Principles of Annex VI to Directive 91/414/EEC, and where this assessment does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern where the assessment at a higher tier level could not be finalised due to a lack of information, and where the assessment performed at the lower tier level does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

5. The database was insufficient to set reference values and the operator, worker, bystander and consumer risk assessment is inconclusive.

6. When the uncertainty in the available input parameters used in groundwater exposure modelling (that are QSAR estimates) is accounted for in the modelling, this modelling for the representative use, indicates a high potential for groundwater contamination by the pesticide active substance carvone, under the geoclimatic conditions represented by all 9 FOCUS groundwater scenarios.

9.3. Overview of the concerns for each representative use considered

(If a particular condition proposed to be taken into account to manage an identified risk, as listed in section 8, has been evaluated as being effective, then 'risk identified' is not indicated in this table.)

All columns are grey as the technical material specification proposed could not be compared to the material tested.

Representative use		Post harvest treatment of potatoes in store including seed potatoes
Operator risk	Risk identified	
	Assessment not finalised	X ⁵
Worker risk	Risk identified	
	Assessment not finalised	X ⁵
Bystander risk	Risk identified	
	Assessment not finalised	X ⁵
Consumer risk	Risk identified	
	Assessment not finalised	X ⁵
Risk to wild non target terrestrial vertebrates	Risk identified	
	Assessment not finalised	X ⁴
Risk to wild non target terrestrial organisms other than vertebrates	Risk identified	
	Assessment not finalised	X ^{1,2,3,4}
Risk to aquatic organisms	Risk identified	
	Assessment not finalised	X ^{1,2,3,4}
Groundwater exposure active substance	Legal parametric value breached	X ⁶
	Assessment not finalised	X ^{1,2}
Groundwater exposure metabolites	Legal parametric value breached	
	Parametric value of 10µg/L ^(a) breached	
	Assessment not finalised	X ³

The superscript numbers in this table relate to the numbered points indicated in sections 9.1 and 9.2

Where there is no superscript number see sections 2 to 6 for further information

(a): Value for non relevant metabolites prescribed in SANCO/221/2000-rev 10-final, European Commission, 2003

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APPENDICES

APPENDIX A – LIST OF END POINTS FOR THE ACTIVE SUBSTANCE AND THE REPRESENTATIVE FORMULATION

Identity, Physical and Chemical Properties, Details of Uses, Further Information

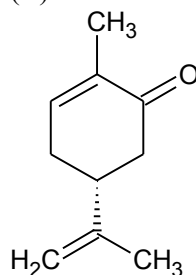
Active substance (ISO Common Name) ‡	Spearmint oil
Function (<i>e.g.</i> fungicide)	Plant Growth Regulator
Rapporteur Member State	Sweden
Co-rapporteur Member State	Not assigned
Identity (Annex IIA, point 1)	
Chemical name (IUPAC) ‡	IUPAC-name not applicable for spearmint oil. For main constituent (R)-carvone : (R)-5-isopropenyl-2-methylcyclohex-2-en-1-one For the other constituents: See the EFSA conclusion
Chemical name (CA) ‡	For spearmint oil : Oil, spearmint For main constituent (R)-carvone : (R) 2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-one For the other constituents: See the EFSA conclusion
CIPAC No ‡	908
CAS No ‡	For spearmint oil: 8008-79-5 For main constituent (R)-carvone :6485-40-1
EC No (EINECS or ELINCS) ‡	No number for Spearmint oil (Spearmint extract 283-656-2, CAS-number 84696-51-5) For main constituent (R)-carvone : 229-352-5
FAO Specification (including year of publication) ‡	Not available.
Minimum purity of the active substance as manufactured ‡	See EFSA conclusion.
Identity of relevant impurities (of toxicological, ecotoxicological and/or environmental concern) in the active substance as manufactured	No relevant impurities.
Molecular formula ‡	For spearmint oil: Not applicable For main constituent (R)-carvone : C ₁₀ H ₁₄ O
Molecular mass ‡	Not applicable for spearmint oil

Structural formula ‡

For main constituent (R)-carvone : 150.21 g/mol

Not applicable for Spearmint oil

(R)-Carvone main constituent of Spearmint oil



For the other components see the EFSA conclusion

Physical and chemical properties (Annex IIA, point 2)

Melting point (state purity) ‡	Open
Boiling point (state purity) ‡	Open
Temperature of decomposition (state purity)	Open
Appearance (state purity) ‡	Open
Vapour pressure (state temperature, state purity) ‡	Open
Henry's law constant ‡	Open
Solubility in water (state temperature, state purity and pH) ‡	Open
Solubility in organic solvents ‡ (state temperature, state purity)	Open
Surface tension ‡ (state concentration and temperature, state purity)	Open
Partition co-efficient ‡ (state temperature, pH and purity)	log P _{ow} is 2.4 for carvone. (No data on Spearmint oil) Not available. Data gap for the individual substances included in the specification of spearmint oil Open
Dissociation constant (state purity) ‡	Open
UV/VIS absorption (max.) incl. ϵ ‡ (state purity, pH)	Open
Flammability ‡ (state purity)	<u>Flammability</u> : Not applicable as spearmint oil is a liquid. <u>Flash point</u> : 92.0 °C for spearmint oil.
Explosive properties ‡ (state purity)	Open
Oxidising properties ‡ (state purity)	Open

SUMMARY OF GOOD AGRICULTURAL PRACTICES FOR PESTICIDE USES

(Application on agricultural and horticultural crops)

XEDA INTERNATIONAL S.A.
2 Zone Artisanale de la Crau
13670 SAINT-ANDIOL, FRANCE

Date : 18 May 2007
Page : 1
Country : EU Member States

Pesticide(s) (common name(s)) : SPEARMINT OIL
EEC, CIPAC and CCPR No(s). : 283-656-2, not applicable and not applicable
Trade name(s) : BIOX-M
Main uses e.g. insecticide, : Plant growth regulator (sprout inhibitor)
fungicide
Applicant : XEDA INTERNATIONAL S.A.

Use Pattern

1	2	3	4		5			6			7			8	9	
Crop and / or situation	F or G	Pest or group of pests controlled	Formulation		Application			Application rate per treatment			PHI (days)	Remarks:				
			Type	Conc. of a.i.	method, kind	growth stage	number (range)	kg a.i./hl	water l/ha	kg a.i./ha						
(a)	(b)	(c)	(d - f)	(i)	(f - h)	(j)					(k)	(l)				
Potato	G*	Sprouting	HN	949 g/L	Hot fogging	Harvested product (BBCH 99)	1-11	94.9	Not applicable	Not applicable	Not applicable	*Indoor storage post-harvest undiluted treatment (not glasshouse)				

Remarks: (a) In case of group of crops the Codex classification should be used
(b) Outdoor or field use (F), or glasshouse application (G)

- (c) e.g. biting and sucking insects, soil born insects, foliar fungi
- (d) e.g. wettable powder (WG), emulsifiable concentration (EC), granule (GR)
- (e) Use CIPAC/FAO Codes where appropriate
- (f) All abbreviations used must be explained
- (g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
- (h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants
- (i) g/kg or g/l
- (j) Growth stage at last treatment
- (k) PHI = Pre-harvest interval
- (l) Remarks may include: Extent of use/economic importance/restrictions (e.g. feeding, grazing)/minimal intervals between applications

Methods of Analysis

Analytical methods for the active substance (Annex IIA, point 4.1)

Technical as (analytical technique)	GC-MS (individual substances included in the specification of spearmint oil)
Impurities in technical as (analytical technique)	Not applicable to spearmint oil extract.
Plant protection product (analytical technique)	Technical as and plant protection product is identical Additionally a validated GC/FID method of for analysis of R-carvone by external calibration is available

Analytical methods for residues (Annex IIA, point 4.2)

Residue definitions for monitoring purposes

Food of plant origin, method 1	Open
Food of plant origin, method 2	Open
Food of animal origin	Open
Soil	Open
Water surface	Open
drinking/ground	Open
Air	Open

Monitoring/Enforcement methods

Food/feed of plant origin (analytical technique and LOQ for methods for monitoring purposes)	Open
Food/feed of animal origin (analytical technique and LOQ for methods for monitoring purposes)	Open
Soil (analytical technique and LOQ)	Open
Water (analytical technique and LOQ)	Open
Air (analytical technique and LOQ)	Open
Body fluids and tissues (analytical technique and LOQ)	Not required as spearmint oil is not classified as T or T ⁺

Classification and proposed labelling with regard to physical and chemical data (Annex IIA, point 10)

RMS/peer review proposal

Active substance

None

Impact on Human and Animal Health

Absorption, distribution, excretion and metabolism (toxicokinetics) (Annex IIA, point 5.1)

Rate and extent of oral absorption ‡	No data available with Spearmint oil, only data from studies with (R)-carvone: Rapid and virtually complete
Distribution ‡	No data available with Spearmint oil
Potential for accumulation ‡	No data available with Spearmint oil
Rate and extent of excretion ‡	No data available with Spearmint oil
Metabolism in animals ‡	No data available with Spearmint oil
Toxicologically relevant compounds (animals and plants) ‡	No data available with Spearmint oil
Toxicologically relevant compounds (environment) ‡	No data available with Spearmint oil

Acute toxicity (Annex IIA, point 5.2)

Rat LD ₅₀ oral ‡	>2000 mg kg ⁻¹	
Rat LD ₅₀ dermal ‡	>2000 mg kg ⁻¹	
Rat LC ₅₀ inhalation ‡	>5.43 mg/L	
Skin irritation ‡	No	
Eye irritation ‡	Slightly irritant	
Skin sensitisation ‡	Sensitiser	R43

Short term toxicity (Annex IIA, point 5.3)

Target / critical effect ‡	No data available with Spearmint oil	
Relevant oral NOAEL ‡	No data available on Spearmint oil.	
Relevant dermal NOAEL ‡	No data available with Spearmint oil	
Relevant inhalation NOAEL ‡	No data available with Spearmint oil	

Genotoxicity ‡ (Annex IIA, point 5.4)

No data available on Spearmint Oil.	

Long term toxicity and carcinogenicity (Annex IIA, point 5.5)

Target/critical effect ‡	No studies are available on Spearmint oil.
Relevant NOAEL ‡	No data available with Spearmint oil

Carcinogenicity ‡

No data available with Spearmint oil	

Reproductive toxicity (Annex IIA, point 5.6)

Reproduction toxicity

Reproduction target / critical effect ‡

No studies are available on Spearmint oil or carvone.	

Relevant parental NOAEL ‡

No data available with Spearmint oil	

Relevant reproductive NOAEL ‡

No data available with Spearmint oil	

Relevant offspring NOAEL ‡

No data available with Spearmint oil	

Developmental toxicity

Developmental target / critical effect ‡

No data available with Spearmint oil	

Relevant maternal NOAEL ‡

No data available with Spearmint oil	

Relevant developmental NOAEL ‡

No data available with Spearmint oil	

Neurotoxicity (Annex IIA, point 5.7)

Acute neurotoxicity ‡

No data available with Spearmint oil	

Repeated neurotoxicity ‡

No data available with Spearmint oil	

Delayed neurotoxicity ‡

No data available with Spearmint oil	

Other toxicological studies (Annex IIA, point 5.8)

Mechanism studies ‡

No data available - not required	

Studies performed on metabolites or impurities ‡

No conclusion could be reached for the toxicological profile of an other active substance constituent (listed in row 2 in Table C.1.2-1 of the Addendum to Volume 4 of the DAR)	

Medical data ‡ (Annex IIA, point 5.9)

No data available with Spearmint oil Contact allergy to carvone flavouring in toothpastes has been reported.	

Summary (Annex IIA, point 5.10)

	Value	Study	Safety factor
ADI ‡	Database insufficient		
AOEL ‡	Database insufficient		
ARfD ‡	Database insufficient		

Dermal absorption ‡ (Annex IIIA, point 7.3)

Spearmint oil	Default values of 100% for dermal absorption

Exposure scenarios (Annex IIIA, point 7.2)

Operator	Spearmint oil An operator using PPE is exposed to 0.06 mg spearmint oil/kg bw/day, when 20L containers are used and 0.01 mg/kg bw/day when 5L containers are used.
Workers	Spearmint oil Working with anti sprouting potatoes will expose the worker with 0.0008 mg/kg bw/day
Bystanders	Spearmint oil Not relevant due to exclusive indoor use.

Classification and proposed labelling with regard to toxicological data (Annex IIA, point 10)

Spearmint oil	RMS/peer review proposal
	Xn Harmful R43 May cause sensitization by skin contact R65 May cause lung damage if swallowed

Metabolism in plants (Annex IIA, point 6.1 and 6.7, Annex IIIA, point 8.1 and 8.6)

Plant groups covered	No data available (data gap)
Rotational crops	Not relevant due to exclusive indoor use
Metabolism in rotational crops similar to metabolism in primary crops	Not applicable
Processed commodities	Not tested
Residue pattern in processed commodities similar to residue pattern in raw commodities	Not applicable
Plant residue definition for monitoring	Possibly (R)-carvone, to be confirmed by further data
Plant residue definition for risk assessment	Open (data gap)
Conversion factor (monitoring to risk assessment)	Currently not applicable

Metabolism in livestock (Annex IIA, point 6.2 and 6.7, Annex IIIA, point 8.1 and 8.6)

Animals covered	GAP does not include feeding restriction, livestock exposure trigger value exceeded → to be addressed further (data gap)
Time needed to reach plateau concentration in milk and eggs	Currently-not applicable
Animal residue definition for monitoring	Currently-not applicable
Animal residue definition for risk assessment	Currently-not applicable
Conversion factor (monitoring to risk assessment)	Currently-not applicable
Metabolism in rat and ruminant similar (yes/no)	Currently-not applicable
Fat soluble residue: (yes/no)	No data on Spearmint oil log P_{ow} is 2.4 for carvone. log P_{ow} may be >3 for other components of spearmint oil and metabolites of R-carvone.

Residues in succeeding crops (Annex IIA, point 6.6, Annex IIIA, point 8.5)

Not relevant due to exclusive indoor use post-harvest

Stability of residues (Annex IIA, point 6 introduction, Annex IIIA, point 8 introduction)

New study available but not eligible for peer review (data gap)

Residues from livestock feeding studies (Annex IIA, point 6.4, Annex IIIA, point 8.3)

Intakes by livestock ≥ 0.1 mg/kg diet/day:	Ruminant: Yes	Poultry: Yes	Pig: Yes
Metabolism studies indicate potential level of residues ≥ 0.01 mg/kg in edible tissues (yes/no)	Not available	Not available	Not available
	Residue levels in feeding studies (dose level: mg/kg)		
	Mean (max) mg/kg		
Muscle	n/a	n/a	n/a
Liver	n/a	n/a	n/a
Kidney	n/a	n/a	n/a
Fat	n/a	n/a	n/a

Milk	n/a	n/a	n/a
Eggs	n/a	n/a	n/a
N/R=Not			require

Summary of critical residues data (Annex IIA, point 6.3, Annex IIIA, point 8.2)

Crop	Northern or Mediterranean Region, field or glasshouse, and any other useful information	Trials results relevant to the representative uses (a)	Recommendation/comments	MRL estimated from trials according to the representative use	HR (c)	STMR (b)
Potato	Indoor, in storage	Waiting period 0 days *; analyte R-carvone ** [mg/kg]: 1.11, 1.19, 1.84 Waiting period 7±1 days *; analyte R-carvone ** [mg/kg]: 0.56, 0.57, 2x 0.93, 1.22, 1.49	* Waiting period/ post-treatment-interval not defined in the GAP table. ** Residue definition for monitoring and risk assessment currently not agreed on Total number of residue trials insufficient (data gap)	open	open	open

(a) Numbers of trials in which particular residue levels were reported *e.g.* 3 x <0.01, 1 x 0.01, 6 x 0.02, 1 x 0.04, 1 x 0.08, 2 x 0.1, 2 x 0.15, 1 x 0.17

(b) Supervised Trials Median Residue *i.e.* the median residue level estimated on the basis of supervised trials relating to the representative use

(c) Highest residue

Consumer risk assessment (Annex IIA, point 6.9, Annex IIIA, point 8.8)

ADI	Agreed toxicological reference values not available
TMDI (% ADI)	Risk assessment not finalised
TMDI (% ADI) according to national (to be specified) diets	Risk assessment not finalised
IEDI (%ADI)	Currently not applicable
NEDI (specify diet) (% ADI)	No data available
Factors included in IEDI and NEDI	Not applicable
ARfD	Agreed toxicological reference values not available
IESTI	Risk assessment not finalised
NESTI (% ARfD) according to national (to be specified) large portion consumption data	Risk assessment not finalised
Factors included in NESTI	Not applicable

⁵ To be done on the basis of WHO guidelines and recommendations with the deviations within the EU so far accepted diets.

Processing factors (Annex IIA, point 6.5, Annex IIIA, point 8.4)

Crop/processed crop	Number of studies	Transfer factor	% Transference *
Currently no data available, possibly to be addressed further depending on the outcome of consumer risk assessment			

Proposed MRLs (Annex IIA, point 6.7, Annex IIIA, point 8.6)

Potatoes

Open (pending monitoring residue definition and complete set of residue trials)

When the MRL is proposed at the LOQ this should be annotated by an asterisk after the number.

Route of degradation (aerobic) in soil (Annex IIA, point 7.1.1.1)

Mineralization after 100 days ‡	No data available.
Non-extractable residues after 100 days ‡	No data available.
Metabolites requiring further consideration ‡ - name and/or code, % of applied (range and maximum)	No data available.

Route of degradation in soil - Supplemental studies (Annex IIA, point 7.1.1.2)

Anaerobic degradation ‡	
Mineralization after 100 days	No data available.
Non-extractable residues after 100 days	No data available.
Metabolites that may require further consideration for risk assessment - name and/or code, % of applied (range and maximum)	No data available.
Soil photolysis ‡	
Metabolites that may require further consideration for risk assessment - name and/or code, % of applied (range and maximum)	No data available.

Soil adsorption/desorption (Annex IIA, point 7.1.2,)

Anaerobic degradation ‡	
K_f/K_{oc}	No data available.
K_d	No data available.
pH dependence (yes/no) (if yes type of dependence)	No data available.

Mobility in soil (Annex IIA, point 7.1.3, Annex IIIA, point 9.1.2)

Column leaching	No data available.
Age residues leaching	No data available.

Lysimeter/ field leaching studies	Not submitted, not required

PEC (soil) (Annex IIIA, point 9.1.3)

Carvone

Method of calculation

Calculation assuming that potatoes with residue levels of 1.84 mg (R)-carvone/kg potato are planted out and that the (R)-carvone is left in the top 5 cm of soil.

Soil DT₅₀ based on QSAR estimation including an assessment factor of 10: 300 days

Application data

Plantation rate of seed tubers: 7.5 tonnes/ha
Application rate: 13.8 g (R)-carvone/ha

PEC_(s)

(mg/kg)

Carvone

Initial

Single application Actual	Single application Time weighted average	Multiple application Actual	Multiple application Time weighted average
0.0185	-	0.032	-

Route and rate of degradation in water (Annex IIA, point 7.2.1)

Hydrolysis of active substance and relevant metabolites (DT₅₀) (state pH and temperature)

Not available. Data gap for the individual substances included in the specification of spearmint oil according to Sanco/10472/2003-rev.5

Photolytic degradation of active substance and relevant metabolites

Not available. Data gap for the individual substances included in the specification of spearmint oil according to Sanco/10472/2003-rev.5

Readily biodegradable (yes/no)

No data available

Degradation in - DT₅₀ water

No data available

water/sediment - DT₉₀ water

- DT₅₀ whole system

- DT₉₀ whole system

Mineralization

No data available

Non-extractable residues

No data available

Distribution in water / sediment systems (active substance)

No data available

Distribution in water / sediment systems (metabolites)

No data available

PEC (surface water) and PEC sediment (Annex IIIA, point 9.2.3)

Method of calculation

FOCUS Step 2. Input data based on QSAR

Application rate

estimates including an assessment factor of 10:
DT₅₀ soil 300 days
DT₅₀ water/sediment 1000 days
K_{oc} 12.4

Calculation assuming that potatoes with residue levels of 1.84 mg (R)-carvone /kg potato are planted out.

Plantation rate of seed tubers: 7.5 tonnes/ha

Application rate: 13.8 g/ha

Season of application March to May.

Main routes of entry

Run-off and drainage (4% for S Europe 2% for S Europe) option no spray drift selected as the use is a seed treatment.

S Europe

PEC_(sw)
(µg/L)
Carvone

Single application Global maximum	Single application Time weighted average	Multiple application Actual	Multiple application Time weighted average
Initial	1.793	-	-

N Europe

PEC_(sw)
(µg/L)
Carvone

Single application Global maximum	Single application Time weighted average	Multiple application Actual	Multiple application Time weighted average
Initial	0.897	-	-

S Europe

PEC_(sed)
(µg/kg)
Carvone

Single application Global maximum	Single application Time weighted average	Multiple application Actual	Multiple application Time weighted average
Initial	0.222	-	-

N Europe

PEC_(sed)
(µg/kg)
Carvone

Single application Global maximum	Single application Time weighted average	Multiple application Actual	Multiple application Time weighted average
Initial	0.111	-	-

PEC (ground water) (Annex IIIA, point 9.2.1)

Method of calculation and type of study (e.g. modelling, field leaching, lysimeter)

FOCUS PEARL v4.4.4
Input data based on QSAR estimates including an assessment factor of 10:
DT₅₀ soil 300 days
K_{oc} 12.4
Q10= 2.58, Walker equation coefficient 0.7.

Application rate

Calculation assuming that potatoes with residue levels of 1.84 mg (R)-carvone /kg potato are planted out and that the (R)-carvone is applied at the soil surface (best case, relative to defining an incorporation depth).
Plantation rate of seed tubers: 7.5 tonnes/ha
Application rate: 13.8 g (R)-carvone/ha
Data of application 5 days before the date of emergence defined for each scenario.

PEC(gw) - FOCUS modelling results (80th percentile annual average concentration at 1m)

FOCUS PEARL 4.4.4 /potato	Scenario	Carvone (µg/L)	Metabolite (µg/L)
			1
	Chateaudun	1.339	-
	Hamburg	0.769	-
	Jokioinen	0.827	-
	Kremsmunster	1.461	-
	Okehampton	0.975	-
	Piacenza	0.695	-
	Porto	0.686	-
	Sevilla	0.555	-
	Thiva	0.880	-

Fate and behaviour in air (Annex IIA, point 7.2.2, Annex III, point 9.3)

Direct photolysis in air

Estimated carvone atmospheric half-life 1.5-3 h (AOP Program v1.92)

Assumed OH radicals' concentration: 0.5×10^6 OH/cm³ (24-hr day value)

Estimated atmospheric half-life 0.884 h (AOP Program v1.92) for the active substance constituent listed in row 2 in Table C.1.2-1 of the Addendum to Volume 4 of the DAR

	Assumed OH radicals' concentration: 1.5×10^6 OH/cm ³ (12-hr day value)
Quantum yield of direct phototransformation	No data
Photochemical oxidative degradation in air	Latitude: Season: DT ₅₀ : 2 hours
Volatilization	No data

PEC (air)

Method of calculation	Estimation based on short half-life.

PEC_(a)

Maximum concentration	Negligible.

Residues requiring further assessment

Environmental occurring residues requiring further assessment by other disciplines (toxicology and ecotoxicology) and or requiring consideration for groundwater exposure.

Soil, Surface Water, Sediment, Ground water, Air: carvone (expected to (R)-carvone)
other less prominent constituents, see Addendum to Volume 4 of the DAR for further details

Monitoring data, if available (Annex IIA, point 7.4)

Soil (indicate location and type of study)	No data available
Surface water (indicate location and type of study)	No data available
Ground water (indicate location and type of study)	No data available

study)

Air (indicate location and type of study)

No data available

Points pertinent to the classification and proposed labelling with regard to fate and behaviour data

Candidate for R53, Not readily biodegradable.

Effects on terrestrial vertebrates (Annex IIA, point 8.1, Annex IIIA, points 10.1 and 10.3)

Acute toxicity to mammals	LD ₅₀ > 2000 mg/kg (rat)
Acute toxicity to birds	No data available
Dietary toxicity to birds	No data available
Reproductive toxicity to birds	No data available

Toxicity/exposure ratios for terrestrial vertebrates (Annex IIIA, points 10.1 and 10.3)

Application rate (kg as/hL)	Crop	Category	Time-scale	TER	Annex VI Trigger
		Birds and mammals		Data gap	

Toxicity data for aquatic species (most sensitive species of each group)

(Annex IIA, point 8.2, Annex IIIA, point 10.2)

Group	Test substance	Time-scale	Endpoint	Toxicity (mg/l)
Laboratory tests				
Fish (<i>Oncorhynchus mykiss</i>)	(R)-Carvone	96 hour	LC ₅₀	Data gap, QSAR estimation: 20.3-32.9*
Invertebrate (<i>Daphnia magna</i>)	(R)-Carvone	48 hour	EC ₅₀	Data gap, QSAR estimation: 9.59-15.1*
Algae	(R)-Carvone	5 day	EC ₅₀	Data gap, QSAR estimation: 4.58-7.36*
Aquatic plant (<i>Lemna gibba</i>)	-	14 day	IC ₅₀	No data available
Microcosm or mesocosm tests				
Not available				

*no experimental data available, values based on QSAR estimations (ECOSAR v0.99h)

Toxicity/Exposure Ratios for the Most Sensitive Aquatic Organisms (Annex IIIA, Point 10.2)

Application rate (kg as/hL)	Crop	Organism	Time-scale	Distance (m)	TER	Annex VI Trigger
		Fish	Acute	Not relevant	Not provided	100
		Invertebrate	Acute	Not relevant	Not provided	100
		Alga	Acute	Not relevant	Not provided	10

Bioconcentration

Bioconcentration factor (BCF)

Log Kow for Carvone reported as 2.4. Data gap for the individual constituents.

Annex VI Trigger for the bioconcentration factor

100

Clearance time (CT₅₀)
(CT₉₀)

Not relevant. No data available

Level of residues (%) in organisms after the 14 day depuration period

Not relevant. No data available

Effects on honeybees (Annex IIA, point 8.3.1, Annex IIIA, point 10.4)

Acute oral toxicity

No data available

Acute contact toxicity

No data available

Hazard Quotients for Honey Bees (Annex IIIA, Point 10.4)

Application rate (kg as/hL)	Crop	Route	Hazard quotient	Annex VI Trigger
Laboratory tests				
		Oral	Data gap	
		Contact	Data gap	

Field or semi-field tests

No data available

Effects on Other Arthropod Species (Annex IIA, Point 8.3.2, Annex IIIA, Point 10.5)

Species	Stage	Test Substance	Dose (Kg as/ha)	Endpoint	Effect	Annex VI Trigger
Laboratory tests						
<i>Aphidius rhopalosiphi</i>	adult	-	-	LR ₅₀	No data available - Data gap	-
<i>Typhlodromus pyri</i>	protonymph	-	-	LR ₅₀	No data available - Data gap	-

Field or semi-field tests
Not available

Effects on earthworms (Annex IIA, point 8.4, Annex IIIA, point 10.6)

Acute toxicity

No data available

Reproductive toxicity

No data available

Toxicity/Exposure Ratios for Earthworms (Annex IIIA, Point 10.6)

Application rate (Kg as/hL)	Crop	Time-scale	TER	Annex VI Trigger
		Acute	Data gap	
		Long term	Data gap	

Effects on Soil Micro-Organisms (Annex IIA, Point 8.5, Annex IIIA, Point 10.7)

Nitrogen mineralization

No data available - Data gap

Carbon mineralization

No data available - Data gap

Effects on non target plants (Annex IIA, point 8.6, Annex IIIA, point 10.8)

No data available - Data gap

Effects on biological methods for sewage treatment (Annex IIA 8.7)

Test type/organism	Endpoint
Activated sludge	No data available
<i>Pseudomonas</i> sp	No data available

Residue definition (ecotoxicologically relevant compounds)

carvone (expected to (R)-carvone)

[Redacted text block containing multiple lines of blacked-out information]

other less prominent constituents, see Addendum to Volume 4 of the DAR for further details

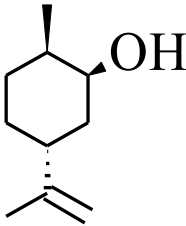
Classification and proposed labelling with regard to ecotoxicological data (Annex IIA, point 10 and Annex IIIA, point 12.3)

Active substance/Product

RMS/peer review proposal (as a „worst case approach’)

N, R50-53, based on the content of the component (listed in row 2 in Table C.1.2-1 of the Addendum to Volume 4 of the DAR) in the five batch analysis but probably can be higher. A read-across to the component listed in row 2 in Table C.1.2-1 of the Addendum to Volume 4 of the DAR is also relevant as (R)-carvone and the above component are structurally similar compounds.

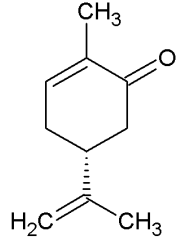
APPENDIX B – USED COMPOUND CODE(S)

Code/Trivial name*	Chemical name	Structural formula
neodihydrocarveol	(1 <i>S</i> ,2 <i>R</i> ,5 <i>R</i>)-2-methyl-5-(prop-1-en-2-yl)cyclohexanol**	

* The metabolite name in bold is the name used in the conclusion.

** ACD/ChemSketch, Advanced Chemistry Development, Inc., ACD/Labs Release: 12.00 Product version: 12.00 (Build 29305, 25 Nov 2008).

APPENDIX C – CONSTITUENTS OF THE ACTIVE SUBSTANCE SPEARMINT OIL

Common name	IUPAC	Min. % w/w	Max. % w/w	Structural formula
(R)-carvone L-carvone	(5 <i>R</i>)-2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one**	55.00	See Addendum to Volume 4 of the DAR (Sweden, 2011)	
Other constituents of spearmint oil*	See Addendum to Volume 4 of the DAR (Sweden, 2011)	See Addendum to Volume 4 of the DAR (Sweden, 2011)	See Addendum to Volume 4 of the DAR (Sweden, 2011)	See Addendum to Volume 4 of the DAR (Sweden, 2011)

*The components of the active substance spearmint oil other than (R)-carvone are not disclosed in the published EFSA conclusion upon advice from the European Commission following a confidentiality claim made by the applicant as provided for in Article 14 of Council Directive 91/414/EEC.

**ACD/ChemSketch, Advanced Chemistry Development, Inc., ACD/Labs Release: 12.00 Product version: 12.00 (Build 29305, 25 Nov 2008)

ABBREVIATIONS

1/n	slope of Freundlich isotherm
λ	wavelength
ε	decadic molar extinction coefficient
°C	degree Celsius (centigrade)
μg	microgram
μm	micrometer (micron)
a.s.	active substance
AChE	acetylcholinesterase
ADE	actual dermal exposure
ADI	acceptable daily intake
AF	assessment factor
AOEL	acceptable operator exposure level
AP	alkaline phosphatase
AR	applied radioactivity
ARfD	acute reference dose
AST	aspartate aminotransferase (SGOT)
AV	avoidance factor
BCF	bioconcentration factor
BUN	blood urea nitrogen
bw	body weight
CAS	Chemical Abstracts Service
CFU	colony forming units
ChE	cholinesterase
CI	confidence interval
CIPAC	Collaborative International Pesticides Analytical Council Limited
CL	confidence limits
cm	centimetre
d	day
DAA	days after application
DAR	draft assessment report
DAT	days after treatment
DM	dry matter
DT ₅₀	period required for 50 percent disappearance (define method of estimation)
DT ₉₀	period required for 90 percent disappearance (define method of estimation)
dw	dry weight
EbC ₅₀	effective concentration (biomass)
EC ₅₀	effective concentration
ECHA	European Chemical Agency
EEC	European Economic Community
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EMDI	estimated maximum daily intake
ER ₅₀	emergence rate/effective rate, median
ErC ₅₀	effective concentration (growth rate)
EU	European Union
EUROPOEM	European Predictive Operator Exposure Model
f(twa)	time weighted average factor
FAO	Food and Agriculture Organisation of the United Nations
FIR	Food intake rate
FOB	functional observation battery
FOCUS	Forum for the Co-ordination of Pesticide Fate Models and their Use

g	gram
GAP	good agricultural practice
GC	gas chromatography
GCPF	Global Crop Protection Federation (formerly known as GIFAP)
GGT	gamma glutamyl transferase
GM	geometric mean
GS	growth stage
GSH	glutathion
h	hour(s)
ha	hectare
Hb	haemoglobin
Hct	haematocrit
hL	hectolitre
HPLC	high pressure liquid chromatography or high performance liquid chromatography
HPLC-MS	high pressure liquid chromatography – mass spectrometry
HQ	hazard quotient
IEDI	international estimated daily intake
IENTI	international estimated short-term intake
ISO	International Organisation for Standardisation
IUPAC	International Union of Pure and Applied Chemistry
JMPR	Joint Meeting on the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues (Joint Meeting on Pesticide Residues)
K _{doc}	organic carbon linear adsorption coefficient
kg	kilogram
K _{Foc}	Freundlich organic carbon adsorption coefficient
L	litre
LC	liquid chromatography
LC ₅₀	lethal concentration, median
LC-MS	liquid chromatography-mass spectrometry
LC-MS-MS	liquid chromatography with tandem mass spectrometry
LD ₅₀	lethal dose, median; dosis letalis media
LDH	lactate dehydrogenase
LOAEL	lowest observable adverse effect level
LOD	limit of detection
LOQ	limit of quantification (determination)
m	metre
M/L	mixing and loading
MAF	multiple application factor
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
mg	milligram
mL	millilitre
mm	millimetre
mN	milli-newton
MRL	maximum residue limit or level
MS	mass spectrometry
MSDS	material safety data sheet
MTD	maximum tolerated dose
MWHC	maximum water holding capacity
NESTI	national estimated short-term intake

ng	nanogram
NOAEC	no observed adverse effect concentration
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOEL	no observed effect level
OM	organic matter content
Pa	pascal
PD	proportion of different food types
PEC	predicted environmental concentration
PEC _{air}	predicted environmental concentration in air
PEC _{gw}	predicted environmental concentration in ground water
PEC _{sed}	predicted environmental concentration in sediment
PEC _{soil}	predicted environmental concentration in soil
PEC _{sw}	predicted environmental concentration in surface water
pH	pH-value
PHED	pesticide handler's exposure data
PHI	pre-harvest interval
PIE	potential inhalation exposure
pK _a	negative logarithm (to the base 10) of the dissociation constant
P _{ow}	partition coefficient between <i>n</i> -octanol and water
PPE	personal protective equipment
ppm	parts per million (10 ⁻⁶)
ppp	plant protection product
PT	proportion of diet obtained in the treated area
PTT	partial thromboplastin time
QSAR	quantitative structure-activity relationship
r ²	coefficient of determination
RPE	respiratory protective equipment
RUD	residue per unit dose
SC	suspension concentrate
SD	standard deviation
SFO	single first-order
SSD	species sensitivity distribution
STMR	supervised trials median residue
t _{1/2}	half-life (define method of estimation)
TER	toxicity exposure ratio
TER _A	toxicity exposure ratio for acute exposure
TER _{LT}	toxicity exposure ratio following chronic exposure
TER _{ST}	toxicity exposure ratio following repeated exposure
TK	technical concentrate
TLV	threshold limit value
TMDI	theoretical maximum daily intake
TRR	total radioactive residue
TSH	thyroid stimulating hormone (thyrotropin)
TWA	time weighted average
UDS	unscheduled DNA synthesis
UV	ultraviolet
W/S	water/sediment
w/v	weight per volume
w/w	weight per weight
WBC	white blood cell
WG	water dispersible granule
WHO	World Health Organisation

wk
yr

week
year



Scientific Committee on Consumer Safety

SCCS

OPINION
on
Fragrance allergens in cosmetic products

The SCCS adopted this opinion at its 15th plenary meeting

of 26-27 June 2012

About the Scientific Committees

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

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

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

SCCS

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

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Summary

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

1. Background

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

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

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

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

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

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

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

Furthermore, two fragrances (natural mixtures) were added

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

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

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

2. Terms of reference

- 1. Does the SCCS still consider that the fragrance allergens currently listed in Annex III, entries 67-92, for labelling purposes represent those fragrance ingredients that the consumer needs to be made aware of when present in cosmetic products?*
- 2. Can the SCCS establish any threshold for their safe use based on the available scientific data?*
- 3. Can the SCCS identify substances where processes (e.g. metabolism, oxidation and hydrolysis) may lead to cross-reactivity and new allergens which are relevant for the protection of the consumer?*

3. Introduction

Fragrance ingredients

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

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

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

4. Clinical aspects of contact allergy to fragrance ingredients

4.1. Spectrum of reactions

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

4.1.1. Allergic contact dermatitis

Mechanism

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

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

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

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

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

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

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

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

Overview of clinical features

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

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

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

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

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

Hands

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

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

Axillae

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

Face

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

4.1.2. Irritant reactions (including contact urticaria)

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

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

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

4.1.3. Pigmentary anomalies

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

4.1.4. Photo-reactions

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

4.1.5. General/respiratory

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

4.2. Patch testing

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

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

4.3. Epidemiology of fragrance allergy

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

A fragrance formula may consist of ten to 300 or more different ingredients. The CosIng database lists 2587 ingredients used for perfuming¹, as well as several other materials classified as odour “masking” agents, which is equivalent with regard to allergy. A mixture of seven fragrance chemicals and one natural extract, which have been identified as major fragrance allergens in the past (34), are used for diagnosing contact allergy to fragrance

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

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

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

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

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

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

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

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

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

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

4.3.2. Clinical epidemiology

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

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

Notwithstanding the potential pitfalls of clinical data, they have proven useful in identifying emerging trends or persisting problems, and also in evaluating the effect of preventive action – either regarding the entire population, or subgroups thereof, such as certain occupations. Regarding the fragrance mixes (FM I and FM II) mentioned above, evidence regarding sensitisation frequencies published since 1999 will be outlined below, thus supplementing the data presented in the SCCNFP opinion on Fragrance Allergy in 1999 (1).

Fragrance Mix I ("Larsen Mix")

Table 4-3: Results with screening agents for contact allergy to fragrance ingredients reported since 1999 in patients patch tested for suspected allergic contact dermatitis in Europe: Fragrance Mix "I" (see Table 4-1). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS (§).

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI)
Sweden (38)	Consecutive patients	2000	3790	6.9
Hungary (39)		1998-1999	3604	8.2 (7.3–9.1) [§]
Czech Republic (40)		1997-2001	12058	5.8 (5.4–6.2) [§]
Ljubljana, Slovenia (41)	Consecutive patients	1989-1998	6129	5.9 (5.3–6.5) [§]
Germany (42)	Consecutive IVDK patients	1996-2002	59298	11.3 (11.0–11.5) [§]
Germany (43)	Consecutive IVDK patients	2005-2008	36961	7.3 (7.0–7.6) [§]
Vienna, Austria (16)	Consecutive patients of one clinic	1997-2000	2660	9.1 (8.1–10.3) [§]
Groningen, Netherlands (44)	Patients (fragrance allergy suspected)	04/2005-06/2007	295	5.8 (3.4–9.1) [§]
The Netherlands (45)	Consecutive patients	09/1998-04/1999	1825	10.6 (9.2–12.1)
The Netherlands (46)	Patients (cosmetic allergy suspected)	1994-1998	757	14.8 (12.3–17.5) [§]
Leuven, Belgium (47)	Consecutive patients	1990-2005	10128	9.1 (8.6–9.7) [§]
Coimbra, Portugal (48)	Consecutive patients	07/1989-06/1999	2600	10.9 (9.7–12.2) [§]
Spain (49)	Consecutive patients	10/2005-06/2008	1253	4.5 (3.4–5.8) [§]
Sheffield, UK (50)	Consecutive patients	1994-1995	744	11.4 (9.2–13.9) [§]
St. John's, London, UK (51)	Consecutive patients	1980-2004	34072	7.7 (7.4–8.0) [§]
Copenhagen, Denmark (52)	Consecutive patients	1985-2007	16173	7.2 (6.8–7.6) [§]
ESSCA (53)	Consecutive patients	2002-2003	9663	7.1 (6.6–7.6) [§]
ESSCA (54)	Consecutive patients	2004	9941	7.6 (7.1–8.2) [§]
ESSCA (55)	Consecutive patients	2005-2006	18542	7.0 (6.6–7.4) [§]

Table 4-4: Results with screening agents for contact allergy to fragrance ingredients reported since 1999 in patients patch tested for suspected allergic contact dermatitis in non-European countries: Fragrance Mix “I” (see Table 4-1). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS (§).

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI)
South Korea (56)	Consecutive patients	04/2002–06/2003	422	9.7 (7.1–13.0) [§]
Lahore, Pakistan (57)	Dermatitis patients	2 years prior to 2002	350	7.7 (5.2–11.0) [§]
Manipal, India (58)	Dermatitis patients	1989-1998	1780	3.1 (2.3–4.0) [§]
Tel Aviv, Israel [§] (59)	Consecutive patients	1999-2000	943	8.5 (6.8–10.5) [§]
Tel Aviv, Israel (60)	Consecutive patients	1998-2004	2156	7.1 (6.1–8.3) [§]
Tehran, Iran (61)	Consecutive patients	2002-2004	250	4.0 (1.9–7.2) [§]
Ankara, Turkey (62)	Consecutive patients	1992-2004	1038	2.1 (1.3–3.2) [§]
Beijing, China (63)	Consecutive patients	2000-2003	378	15.9 (12.3–20.0) [§]
USA (Canada) (64)	Probably consecutive patients	2003	1603	5.9
NACDG 2009 (US and Canada) (65)	Consecutive patients	2005-2006	4439	11.5

Note: § Possibly included in (60).

Beyond the studies discussed above, regarding a time trend of sensitisation to FM I, a significant increase of positive results to FM I until 1998, and a significant drop thereafter has been noted in the IVDK study covering 1996 to 2002 (42). A similar drop from 1999 to 2007 has been observed in female, but not male patients from Copenhagen (52). In accordance with these findings, the prevalence of positive reactions to FM I doubled, or thereabouts, from 1989-1993 to 1994-1998 in Ljubljana, Slovenia (41).

Within Europe, a comparison between different countries and clinical departments is possible. An EEC DRG study covering 1996-2000 found 9.7% positives to FM I (range: 5.0–12.6% in ten departments from seven European countries (66). A different European study, covering 10/1997-10/1998, found 11.3% (95% CI: 9.9–12.9%) positive reactions to FM I in 1,855 patients; the variation between centres was marked: Gentofte 8.2% vs. Leuven 23.0% as extremes (67). In the first study of the European Surveillance System on Contact Allergies (ESSCA), covering 2002 and 2003, 9663 patients were patch tested with FM I, overall yielding 7.1% positive reactions with marked variation between participating departments. In Dortmund, Germany, the minimum frequency of 3.7% was noted, while in Lahti, Finland, the highest prevalence, namely 10.4%, was found (53). Subsequently, in the year 2004, the overall prevalence was 7.6%, i.e. largely unchanged (54). In the most recent study by ESSCA, based on 2005/2006 PT data across Europe, significant differences were again noted, this time on the aggregated level of European regions, with FM I sensitisation being the least frequent in the Southern countries (4.8% [95% CI: 3.9–5.5%] age- and sex-standardised prevalence) vs. 7.7% (95% CI: 7.0–8.4%) in the central European departments, with the Finnish, Polish and Lithuanian departments (5.7% [95%

CI: 4.6 – 6.8%]) and the UK network (6.8% [95% CI: 6.3 – 7.3%]) in an intermediate position (55).

Fragrance Mix II

Table 4-5: Results with screening agents for contact allergy to fragrance ingredients reported since 1999 in patients patch tested for suspected allergic contact dermatitis: Fragrance Mix “II” (see Table 4-2). The FM II was only conceived in 2005, so results are still sparse). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS ^(§).

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI)
EU (35)	Six clinical depts.	10/2002-06/2003	1701	2.9 (2.2–3.9) [§]
IVDK, Germany (68)	Consecutive patients	01/2005-12/2008	35633	4.9 (4.7–5.1) [§]
Groningen, Netherlands (44)	Patients (fragrance allergy suspected)	04/2005-06/2007	227	9.3 (5.8–13.8) [§]
Leuven, Belgium (47)	Consecutive patients	2005 only	335	2.1 (0.8–4.3) [§]
Spain (49)	Consecutive patients	10/2005-06/2008	1253	0.6 (0.2–1.1) [§]
Denmark (69) on behalf of the DCDG, 2010	Consecutive patients	2005-2008	12302	4.5 (4.1–4.9) [§]

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) has been the most frequently reported chemical causing fragrance allergy since the 1999 opinion on fragrance allergy. In total, reports of about 1500 cases have been published in the scientific literature (see section 7.1).

HICC was recognised as an allergen in 1995 (70) and later included in the new perfume mixture, Fragrance Mix II (71), which is routinely used for the diagnosis of perfume allergy, see above. Furthermore, it is recommended to test separately with HICC, because it is a very frequent allergen (37) and detects relevant fragrance sensitisation which would otherwise have been missed (49). In the studies performed in European dermatology clinics, 0.5-2.7% of eczema patients have been found to be allergic to HICC with the highest frequency in central Europe (55). For further details see Table 4-6.

Table 4-6: Results with fragrance contact allergy screening agents reported since 1999 in patients patch tested for suspected allergic contact dermatitis: **HICC** (5% pet. if not stated otherwise). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS ^(§).

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI)
Lithuania (72)	Consecutive patients	04/2006-10/2008	816	0.9 (0.3–1.8) [§]
Spain (49)	Consecutive patients	10/2005-06/2008	852	0.8 (0.3–1.7) [§]
Germany (CH, AT) (73)	Consecutive patients	03/2000-02/2001	3245	1.9 (1.5–2.4) [§]

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI)
Germany (CH, AT) (74)	Consecutive patients	01/2003-12/2004	21325	2.4 (2.2–2.6) [§]
Germany (CH, AT) (68)	Consecutive patients	01/2005-12/2008	35582	2.3 (2.2–2.5) [§]
Belgium (47)	Consecutive patients	2002-2005	2901	2.1 (1.6–2.7) [§]
Denmark (69)	Consecutive patients	2005-2008	12302	2.4 (2.1–2.7) [§]
South Korea (56)	Consecutive patients	04/2002–06/2003	422	1.7 (0.6–3.4) [§]
USA, Canada (64)	Probably consecutive patients	2003	1603	0.4 (0.2–0.9) [§]

Myroxylon pereirae (Balsam of Peru)

Myroxylon pereirae is a balm obtained from a Central American tree. It is used as a screening substance for fragrance allergy in Europe and other geographical areas. Although the crude balm is not used in Europe in cosmetics, extracts and distillates are used (75). This natural mixture has been employed as screening agent in the baseline series for many decades. Hence, a wealth of data is available; Table 4-7 summarises results of the past 10 years.

Table 4-7: Results with fragrance contact allergy screening agents reported since 1999 in patients patch tested for suspected allergic contact dermatitis: ***Myroxylon pereirae* resin** (Balsam of Peru) (25% pet.). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS ([§]).

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI) [§]
Tel Aviv, Israel (59) #	Consecutive patients	1999-2000	943	6.6 (5.1–8.4) [§]
South Korea (56)	Consecutive patients	04/2002 – 06/2003	422	7.3 (5.1–10.3) [§]
Tel Aviv, Israel (60)	Consecutive patients	1998-2004	2156	3.6 (2.9–4.5) [§]
Manipal, India (58)	Dermatitis patients	1989-1998	1780	1.0 (0.5 – 1.5) [§]
Tehran, Iran (61)	Consecutive patients	2002-2004	250	2.4 (0.9–5.2) [§]
Sevilla, Spain (76)	Consecutive patients	2002-2004	863	5.8 (4.3–7.6) [§]
Ankara, Turkey (62)	Consecutive patients	1992-2004	1038	2.1 (1.3–3.2) [§]
Vienna, Austria (16)	Consecutive patients of one clinic	1997-2000	2660	5.4 (4.6–6.3) [§]
Czech Republic (40)	Consecutive patients	1997-2001	12058	7.3 (6.8–7.8) [§]
Spain (49)	Consecutive patients	10/2005-06/2008	1253	6.4 (5.1–7.9) [§]

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI) [§]
Copenhagen, Denmark (52)	Consecutive patients	1985-2007	16173	3.9 (3.6–4.2) [§]
Sweden (38)	Consecutive patients	2000	3790	6.5
Nine European countries (53)	Consecutive patients	2002-2003	9672	6.1
Germany, three Swiss and one Austrian Dept. (43)	Consecutive patients	2005-2008	36919	8.0 (7.7–8.3)
Ten depts. From seven EU countries (66)	Consecutive patients	1996-2000	26210	6.0
USA (Canada) (64)	Probably consecutive patients	2003	1603	6.6
NACDG 2009 (65)	Consecutive patients	2005-2006	4449	11.9

Oil of turpentine

This natural extract is not tested in all baseline series. It is considered as a minor screening allergen for fragrance contact allergy. Moreover, oil of turpentine is used as a raw material in perfumery (see Annex I). Table 4-8 summarises results of the past 10 years with patch testing of consecutive patients.

Table 4-8: Results with fragrance contact allergy screening agents reported since 1999 in patients patch tested for suspected allergic contact dermatitis: **Oil of turpentine** (10% pet.) patients patch tested for suspected allergic contact dermatitis. If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS ([§]).

Country	Population	Year(s)	No. tested	Crude % positive (95% CI) [§]
Lisbon, Portugal (77); virtually no .delta.-3-carene	Consecutive patients	1979-1983	4316	2.3 (1.9–2.8) [§]
Birmingham, UK (78)	Potters with occup. hand dermatitis	6 months; prior to 1996	24	14/4 pos. to "Indonesian turpentine"
Austria/Germany (IVDK) (79)	Consecutive patients	1992-1995	27658	0.47 (0.39–0.55) [§]
Austria/Germany (IVDK) (42)	Consecutive patients	1996-2002	59478	Annual prevalence 1.6 to 4.4%
Augsburg, Germany (80)	Population sample	1998	1141	1.2% (on population level!)
Europe (ESSCA) (53)	Consecutive patients	2002/03	3767	1.6%
Austria/Germany/Switzerland (IVDK) (43)	Consecutive patients	2005-2008	37163	1.8%

An “overall burden” of fragrance contact allergy, in terms of the prevalence of contact allergy to at least one of the up-to-five screening allergens present in the baseline series (FM I, FM II, HICC, *Myroxylon pereirae*, oil of turpentine) has not been given in the published studies. A re-analysis of data from the two published studies of the IVDK (43, 68), covering central Europe from 2005 to 2008 (Germany, Austria and Switzerland), yielded an estimate of such overall prevalence of 16.2% (95% CI: 15.8-16.6%) (IVDK technical report, 2011-11-18).

4.3.3. Population-based epidemiology

In principle, the examination of a representative sample of the population is the most valid approach for estimating disease frequency, as there is no systematic selection process. However, in practice, participation of much less than 70% of those approached introduces the possibility of self-selection and thus of biased morbidity (or risk) estimates. Moreover, the resources needed prohibit regular, e.g. yearly, patch test studies in a sample of several thousand persons. For these reasons few studies exist (see Table 4-9).

A Swedish study of hand eczema in an industrial city showed that among 1,087 individuals recruited from the general population with symptoms of present or previous hand eczema, 5.8% were positive to the Fragrance Mix (81). In Denmark, Fragrance Mix sensitivity was found in 1.1% (0.3-2.1%) of 567 persons drawn as a sample from the general Danish population; only nickel sensitivity was more prevalent (82). In Italy, female patients with hand eczema caused by contact with detergents were patch tested. Of 1100 women, 3.1% reacted to Fragrance Mix I (83). A control group of 619 female patients with no eczema disease were also patch tested; 1.3% were positive to the Fragrance Mix (83). On the other hand, in a sample of 593 healthy Italian recruits, only three positive reactions (0.50%) to FM I were observed (84). Among Danish school children, 14-15 years of age, fragrance contact allergy was detected in 1.8% by patch testing with Fragrance Mix I (85). A study of 85 American student nurses showed that 15 (17.6%) had a positive reaction to Fragrance Mix I; 12 of the individuals also had a positive history of contact dermatitis (86). In this study the concentration of Fragrance Mix I was 16% as opposed to the currently recommended concentration of 8% and the study included only young females. Both of these factors may have contributed to the high prevalence of fragrance sensitivity found.

In 1990, 1998 and 2006, samples of the Danish adult population living in the Copenhagen area were patch tested with the European baseline series. In total 4299 individuals aged 18-69 years (18-41 years only in 1998) completed a pre-mailed questionnaire and were patch tested with FM I and *Myroxylon pereirae* (82, 87, 88). In 1990, 1.1% were found positive to FM I and in 2006, 1.6% were positive, which means no general change. However, when the age group of 18-41 years was analysed, the prevalence of FM I sensitisation followed an inverted V-pattern among women, i.e. an increase from 0.7% in 1990 to 3.9% in 1998, followed by a decrease to 2.3% in 2006. The participation rate varied in the three samples from 71.5% in 1990 to 52.4% in 1998, and to 43.7% in 2006 (82, 87, 88).

Contact sensitisation to FM I is strongly age related, with the relative risk more than doubling in the older age groups, compared to younger PT patients. This has been found in both bivariate (89) and adjusted multifactorial analyses (90). Hence, in older samples of the population, the prevalence of contact allergy to fragrance ingredients in general, and to FM I in particular, can be expected to be higher than in younger samples. From this background, the strikingly high prevalence observed in the MONICA/KORA allergy study in Augsburg, Germany (see Table 4-9) (80), may be explained, together with some residual confounding from the rather complex sampling process.

Table 4-9: Results from patch testing with Fragrance Mix I in different population based groups.

Country (Ref.)	Population	Year(s)	No. tested	% positive (95% CI)
Italy (83)	Females without eczema	Not given	619	1.3
Italy (84)	Male recruits	Not given	593	0.50
Denmark (82)	Population sample adults, 15-69 years	1990-91	567	1.1
Denmark (85)	School children 12-16 years old	1995/96	717	1.8
Denmark (82, 87)	Population sample adults, 18-41 years	Jan-Nov 1998	414	2.7
Denmark (88)	Population sample adults, 18-69 years	June 2006–May 2008	3460	1.6
Norway (91)	Population sample adults, 18-69 years. (Results reported in 2007)	1994 (92)	1236	1.8 (1.1–2.7)
Germany (80)	Subgroup of MONICA sample, age 25-74	1994/95	1141	11.4
USA (86)	Student nurses, females	1980	85	17.6*
Sweden (81)	Population sample adults, age 20-65 years reporting hand eczema	1983-84	1087	5.8*

Note: * Testing performed with Fragrance Mix I, containing 16% allergens; the currently used Fragrance Mix I contains 8% allergens (see above).

Table 4-10: Results from patch testing with other fragrance allergens in different population based groups. If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS ([§]).

Country (Ref.)	Population	Year(s)	Fragrance allergen	No. tested	% positive (95% CI) [§]
Thailand (93)	Convenience sample (via advertisement), age 18-55	Not given	Isoeugenol, <i>Evernia prunastri</i> , <i>Myroxylon pereirae</i> *	2545	Positive to at least one of three allergens: 2.5 (1.9–3.2) [§]
Germany (80)	Subgroup of MONICA sample, age 25-74	1994/95	<i>Myroxylon pereirae</i>	1141	2.4
Denmark (88)	Population sample, age 18-69	1990 2006	<i>Myroxylon pereirae</i>	567 3460	1.1 0.1

Note: * *Myroxylon pereirae* is a balm obtained from a Central American tree. It is used as a screening substance for fragrance allergy in Europe and other geographical areas. Although the crude balm is not used in Europe in cosmetics, extracts and distillates are used (75).

4.4. Consumer products as a cause of fragrance contact sensitisation and allergic contact dermatitis

4.4.1. Clinical relevance

Clinical relevance is a concept used to describe the significance of a positive (allergic) patch test reaction for an individual patient: a reaction is deemed relevant if contact allergy to the substance is associated with previous or current episodes of allergic contact dermatitis. Thereby, the evaluation of clinical relevance links past exposure to morbidity. For the evaluation of relevance, past or recent exposure(s) to the allergen need to be identified in the patient's history. The success of this process generally depends on:

- The patient's understanding and awareness;
- The dermatologist's knowledge concerning exposures;
- Ingredient labelling; and
- Information about the actual chemical composition of the implicated product.

As these requirements may be met to a varying extent, the validity of relevance information as reported in clinical studies may also be variable. However, information on clinical relevance is important, in principle, because the proportion of currently relevant sensitisations reflects the amount of current exposure and resulting disease state, which may increase or decrease with time. In this way, current relevance also reflects the direct burden of a fragrance contact allergy to the individual and indirectly to society. Further important aspects of the evaluation of clinical relevance as a final step of patch testing have been discussed (32, 94-96).

Generally, clinical relevance is categorised as “current”, “previous” or “unknown”. Further differentiation has been introduced by adding information on:

- Occupational versus non-occupational causation; and
- The level of certainty of the relevance statement, e.g. as “certain”, “probable”, “possible”.

In some cases, clinical relevance may not be established due to:

- Immunological cross-reactivity with an individual allergen, diagnosed or not;
- Active sensitisation by the patch testing;
- Contact sensitisation not caused by the substance, but by a contaminating constituent; or
- Failure to test with a true hapten (e.g. haptens formed from prehaptens on exposure to air, see chapter 5).

It should be noted that this statement on clinical relevance refers to the past history of a patient. This implies that a lack of, or unknown, clinical relevance does not make future allergen avoidance unnecessary.

In the context of contact allergy to fragrance ingredients, a number of alternative concepts of relevance have been used, for example:

- A history of intolerance to perfume or to perfumed products;
- A history of intolerance to perfume actually containing the allergen diagnosed;
- Detection of the culprit allergen in a perfume previously used.

4.4.2. Elicitation with clinical symptoms/signs, current and past

In case reports or small series, the clinical relevance of positive patch test reactions is usually well established and presented in detail. Moreover, a few large-scale clinical studies on contact allergy to fragrance ingredients have reported results on clinical relevance, which will be presented and discussed in this section. The studies can be subdivided into those which focus on medical history, patch testing with consumer products or detection of specific allergens in consumer products used by patients.

Medical history

A series of studies conducted in the 1990s showed that most individuals with contact allergy to fragrance ingredients were aware that they could not tolerate fragranced products on their skin and were able to specifically name product categories that initiated their disease (9). In this context, colognes, deodorants and lotions were named significantly more often by fragrance allergic dermatitis patients than by patients without fragrance contact allergy (3). These studies are described in the SCCNFP opinion on fragrance allergy of 1999 (1). Newer studies are outlined below.

NACDG 2009 study (65)

The definition of “present” clinical relevance in this North American network study was strict, requiring:

- A positive use or patch test with the suspected item(s) for “definite” relevance; and
- Verification of the presence of the allergen in known skin contactants, and consistent clinical presentation for “probable”.

If these conditions were not met, but skin contact to items generally containing the item was likely, “possible” was used.

Regarding fragrance allergens, the proportions were as described in Table 4-11.

Table 4-11: Extract from ((65) Table 3) regarding the proportion of patients with “present clinical relevance” (see text) and “past clinical relevance” (criteria not given).

Fragrance allergen	n (tested)	% (pos.)	Current relevance (%)			Past relevance (%)
			Definite	Probable	Possible	
<i>Myroxylon pereirae</i>	4449	11.9	1.3	33	53	2.7
FM I	4439	11.5	2.0	29.4	54.3	4.3
Cinnamal	4435	3.1	1.5	33.8	50	2.9
Ylang-Ylang oil	4434	1.5	4.6	10.8	73.8	1.5
Jasmine absolute	4447	1.1	0	24.5	67.3	6.1

Frosch 2002 (a) study (67)

In this study, 1,855 consecutive patients were patch tested with FM I and a series of a further 14 fragrance chemicals. Prior to the test, the history of adverse reactions to fragrances was classified as “certain” (6.6%), “probable” (8.0%), “questionable” (9.2%) or “none” (76.1%) (see (71)).

Frosch 2002 (b) study (97)

A series of 18 essential oils or components thereof, together with FM I, was assessed in 1,606 consecutive patients. Similar to the above study, the proportions of patients with a “certain” or “probable” history (or otherwise) and positive reactions to either FM I or the

special series, or both, were cross-tabulated. Of note, 53.7% of patients with positive reactions to FM I only, had no history. Similarly 54.2% of patients with positive reactions only to one of the essential oils had no history. However, in cases of reactivity to both FM I and one of the essential oils, the proportion of patients with no history was only 36.5%.

Frosch 2005 study (35)

The diagnostic properties of FM I and the new FM II were evaluated in 1,701 consecutive patients patch tested in six European centres. Contrasting a “certain” (found in 8.7% of patients) with “no history” (75.3% of patients), the sensitivity of FM I was 25.2%, and the positive predictive value (PPV) 45.1%. In comparison, the sensitivity of FM II at 14% concentration was 13.5% and the PPV was 55.6%. The combination of the two mixes was important, as more patients with a “certain” history, but also independently from history, reacted to just one of the mixes rather than to both.

Danish Contact Dermatitis Group 2005-2008 (69)

In 12302 consecutive patients patch tested in seven dermatology clinics and three university hospitals, 10.6% were positive to one or more of the fragrance allergy markers (FM I, FM II, *Myroxylon pereirae* or hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)). Clinical relevance covered current and/or past relevance based on: 1) medical history; 2) results of patch and/or use tests; 3) ingredient labelling; or 4) chemical analysis. Clinical relevance was found in 71.0% of cases positive to FM I, 72.2% of those positive to FM II and 76.7% of those positive to HICC. These proportions were higher than the average for other cosmetic allergens such as preservatives and hair dyes, which gave relevant reactions in about 50% of those positive, as did *Myroxylon pereirae*. *Myroxylon pereirae* itself is not used in cosmetics as it is banned, but sensitisation may be caused by exposures to related substances and thus relevance may be difficult to determine.

Cosmetic products

Fragrance formulae from cosmetic products

Popular fine fragrances (5), as well as toilet soaps, shampoos, lotions, deodorants, and aftershaves have been shown to provoke allergic contact dermatitis in patients when used for patch testing (5, 6, 98, 99). Moreover, commercially available fragrance formulae and dilutions of individual fragrance allergens were potent elicitors of allergic contact dermatitis under simulated use conditions (10, 100, 101).

More recently, deodorants spiked with the fragrance allergens cinnamal, hydroxycitronellal and HICC, respectively, in realistic in-use concentrations were shown to elicit allergic contact dermatitis in 89-100% of the fragrance allergic individuals tested (102-104). In 87.5% of HICC sensitised individuals the use of a cream (and in 82.8% the use of an ethanol solution) spiked with HICC provoked dermatitis (105). These studies are discussed in more detail in chapter 11 on quantitative aspects. Other new studies are mentioned below:

IVDK “own perfumes” study (106)

A different perspective on clinical relevance is provided by assessing the proportion of positive reactions to the FM I or single fragrance allergens in patients who had not tolerated certain perfumed products, such as deodorants and aftershaves and who were patch test positive to these cosmetics. The following two tables are taken from this publication.

Table 4-12: Extract from ((106) Table 2) on the frequency of positive reactions to fragrance allergens in patients with vs. without positive patch test reaction to their own deodorant.

Fragrance allergen	Conc. (%)	Deodorant positive (n=66)		Deodorant negative (n=855)	
		n (test)	% pos. (95% CI)	n (test)	% pos. (95% CI)
Fragrance Mix I	8	61	38.0 (24.1-51.9)	805	15.0 (12.5-17.5)
<i>Myroxylon pereirae</i>	25	60	22.9 (12.7-33.1)	806	9.1 (7.2-11.0)
Hydroxycitronellal	1	33	6.5 (0.7-12.3)	204	4.3 (1.5-7.1)
Isoeugenol	1	33	6.5 (0.7-12.3)	204	7.2 (3.6-10.8)
Cinnamal	1	29	11.3 (0-24.1)	133	1.1 (0-2.7)
Geraniol	1	29	8.3 (0-20.4)	141	0 (0-2.1)

Of the 66 patients with a positive patch test reaction to their own deodorant, most had positive reactions to one or more fragrance allergens. This was much more prevalent than those patients in whom no positive reaction to their deodorant was observed. This observation supports the notion that the respective fragrance allergens are important in contact allergy to fragrance ingredients caused by deodorants, supporting data regarding exposure (chapter 10.1).

Table 4-13: Extract from ((106) Table 2) on the frequency of positive reactions to fragrance allergens in patients with vs. without positive patch test reaction to their own aftershave, eau de toilette or perfume.

Fragrance allergen	Conc. (%)	Product positive (n=63)		Product negative (n=819)	
		n (test)	% pos. (95% CI)	n (test)	% pos. (95% CI)
Fragrance Mix I	8	56	57.1 (46.2-68.1)	764	13.9 (11.4-16.4)
<i>Myroxylon pereirae</i>	25	56	13.9 (7.3-20.4)	766	8.8 (6.8-10.7)
HICC	5	20	58.3 (37.5-79.0)	310	1.3 (0-2.7)
<i>Evernia prunastri</i>	1	28	22.1 (7.0-37.2)	153	8.8 (4.2-13.4)
Hydroxycitronellal	1	33	6.5 (0.7-12.3)	204	4.3 (1.5-7.1)
<i>Cananga odorata</i> (ylang-ylang oil)	10	7	16.3 (2.0-30.5)	43	5.0 (0-11.3)

Similar results were obtained from the subgroup of patients with a positive reaction to their eau de toilette, aftershave (hydroalcohol solutions) or perfumes (Table 4-13). However, notable differences were: (i) the greater relative importance of *Evernia prunastri* (Oak moss absolute); and (ii) generally an extremely high proportion of positive reactions to various other fragrance ingredients.

4.4.3. Elicitation in diagnostic patch tests without clinical history

In a variable proportion of patients, a positive patch test reaction does not correlate with recent or past episodes of presumptive allergic contact dermatitis. Apart from particular circumstances, such as cross-reactivity or reactivity to contaminants outlined above, there are several possible explanations for this:

- The patch test reaction was a false-positive (irritant).
- There was erroneous recall/interpretation of the patient's history (false-negative).
- Lack of knowledge concerning exposures.

- If the patient is weakly sensitised (e.g. by a low induction dose), the occlusive exposure during patch testing may have been the only exposure above the individual elicitation threshold capable of eliciting an unequivocal allergic contact reaction. In this situation, clinical relevance would be classified as “unknown”. Nevertheless, there is an alteration of the immune status of the individual.

Sometimes, a repeated open application or provocative use test is employed to mimic “normal” exposure to the allergen. A positive reaction to such a use-related test confirms actual sensitisation. Moreover, the positive result supports the necessity of future allergen avoidance. Apart from the risk of developing allergic contact dermatitis in the future, sensitisation means an alteration of the immune status of the individual.

4.5. Socio-economic impact of contact allergy

4.5.1. Health related quality of life

Skin diseases in general are known to affect quality of life significantly (107); this also applies to eczema, where most studies concern atopic dermatitis and hand eczema patients (108, 109). Hand eczema has a poor prognosis and may affect the self-image, limit social activities and lead to occupational restrictions (109, 110). The quality of life in hand eczema patients with fragrance contact allergy is affected in a similar degree as patients with other contact allergies (111).

In a questionnaire study of 117 patients recently diagnosed with contact allergy to fragrance ingredients, most presented with hand or facial eczema. In response to the question if and how fragrance allergy had affected their life situation, 67.5% replied that they often had to take special precautions, 47.0% replied that they were often bothered by eczema and itch, 17.1% said that they had had to take sick leave due to their fragrance contact allergy and 45.3% felt that fragrance contact allergy had significantly influenced their daily living (112).

4.5.2. Occupational restrictions

Contact allergy is known to influence severity and prognosis of hand eczema (113, 114) including risk of sick leave (111). Fragrance contact allergy is mostly of a non-occupational origin (90) related to the personal use of scented cosmetics, but may have secondary occupational consequences. This may be due to exposure to fragrance ingredients also in the work place or because hand eczema has developed. Hand eczema itself may make it impossible to remain in the trade even if protective equipment is used. In young people, fragrance allergy may limit the choice of occupations, as it will be difficult to work as a hairdresser, cosmetologist or in other occupations with a significant skin exposure to fragranced products.

4.5.3. Costs to health care/health economics

In a population based study of 3,460 individuals, contact allergy to FM I was found in 1.6%; logistic regression analyses showed that medical consultation due to cosmetic dermatitis (OR 3.37, 95% CI 1.83-6.20) and cosmetic dermatitis within the past 12 months (OR 3.53, CI 2.02-6.17) were significantly associated with sensitisation to FM I (88). Further, as mentioned above, fragrance allergy may lead to sick leave (112). No specific cost estimates for fragrance allergy exist, but the yearly total costs of contact dermatitis in Western Europe was estimated to be 5.2 billion Euro in 1997. Prices were based on the Allergy White Paper (1997) and on results of investigations and extrapolations of known data for Western Europe (115). Fragrance allergy is the second most frequent cause of contact allergy after nickel allergy and is seen in every 10th patient investigated for contact allergy. Even a modest reduction in nickel allergy has been estimated to have the value of 12 million Euro/year/million people in Denmark (Environmental Project Nr. 929, 2004; <http://www2.mst.dk/Udgiv/publications/2004/87-7614-295-7/pdf/87-7614-296-5.pdf>, last

accessed 2011-11-13). The costs are likely to differ in other countries, some with higher expenses and some with lower costs. These estimates show that the cost of contact allergy in the population may be considerable.

4.6. Allergen avoidance

Generally, “allergen avoidance” can be regarded as having two aspects: (i) primary prevention of the acquisition of contact allergy achieved by avoiding or limiting exposure of the general population, or certain parts of it, to allergens; and (ii) secondary prevention in terms of avoiding (re-)elicitation of allergic contact dermatitis in sensitised individuals.

4.6.1. Primary prevention: limiting or eliminating exposure to allergens in the population

The main aim of public health is the primary prevention of disease in populations. Allergic contact dermatitis (to fragrances) has the potential to have a significant impact on quality of life, including effects on fitness for work (chapter 4.5). Moreover, it is a common phenomenon and therefore a reduction of exposure to (fragrance) allergens must be an objective of effective Public Health measures.

Means of limiting or eliminating exposure to fragrance allergens include the following:

- *Prohibition* by regulatory measures or other means.
- *Restriction* by regulatory measures or other means of the maximum permissible concentration of a substance, or a critical component of natural mixtures, possibly according to different uses and product types, respectively.
- *Substitution* with suitable, but less or non-allergenic compounds. Substitution by a component which is chemically different, but effectively not different in terms of allergenicity or cross-reactivity, is not adequate (e.g. an ester) (chapter 5).
- *Formulating the fragrance* with the aim of limiting or eliminating those substances for which a sensitising potential has been shown. One difficulty with this approach is that sometimes no sensitisation data exist for those components of a fragrance formula which are used to replace a “known sensitiser”.
- *Deliberate avoidance* of the use of fragrances where they are not essential to the function of a finished product, but used merely to add to its appeal. Examples could include most cosmetics, topical medicaments, detergents etc., but obviously not perfumes, eau de toilette and other products used for their scent.
- *Information, e.g. labelling* so that the consumer may make an informed choice to avoid exposure to a particular ingredient.

4.6.2. Secondary prevention: avoiding re-exposure to (a) specific sensitiser(s) in clinically diagnosed individuals

In clinical dermatology, avoidance of re-exposure to an allergen is central to the care of sensitised patients. Contact sensitisation, as a latent condition, persists life-long, and therefore allergen avoidance is the only means of avoiding potentially severe and/or handicapping disease, which affects quality of life and may affect fitness for work, i.e. allergic contact dermatitis.

In this context, the valid diagnosis of sensitisation, by patch testing (32) with standardised materials, is a prerequisite of successful allergen avoidance.

In the case of fragrances, a history clearly indicative of “fragrance dermatitis” but in which patch testing with commercially available test preparations is negative, most probably reflects a shortcoming of the patch test procedure, namely, a false-negative investigation. An important cause is inadequate information on the presence of fragrance substances

present in cosmetic products (and consumer products in general). This means that patients cannot be tested for relevant substances.

A false-negative investigation can also be due to a number of other reasons: (i) non-adherence to scientific recommendations (32) or guidelines (e.g. (116)); (ii) sub-optimal patch test concentration; or (iii) use of non-oxidised material if oxidised material is the true allergen.

In an “ideal” case, from the point of view of successful patient management, the test procedure identifies all the allergen(s) to which the patient has developed contact allergy, according to the information on the culprit product(s) brought in by the patient. Such contact sensitisation is termed “clinically relevant” (65), and the need for allergen avoidance in the future is unequivocally evident in these cases. However, not infrequently, clinical relevance of an allergic patch test reaction cannot be ascertained for various reasons, which may be beyond control by the clinician (see chapter 4.4). Nevertheless, future elicitation of allergic contact dermatitis by sufficient contact with the identified “non-relevant” allergen may be expected. Hence, the patient will need to avoid the respective substance(s).

In a less “ideal” case, only part of the fragrance allergens having caused allergic contact dermatitis are identified (and can subsequently be avoided), while another part remains unidentified, for instance because it is: (i) not labelled on the product; and/or (ii) not available for routine diagnostic patch testing (special investigations such as chemical analysis of the culprit product, and break-down patch testing of its individual components, are performed rarely). Such “residual” undetermined sensitisation will hamper the success of secondary prevention of allergic contact dermatitis due to fragrances.

The above consideration raises the question for the patient of how to identify fragrance chemicals in cosmetics and other products coming into contact with the skin, such as detergents and household products, topical medicaments, products used professionally (e.g. by hairdressers, beauticians, masseurs, aromatherapists), and in other industrially used categories of products (7) (see also chapter 9). In this regard, the labelling with “perfume” or “contains fragrances” does not provide sufficient information. Moreover, such general labelling has two main disadvantages:

- It does not aid the identification of past exposure to specific agents when planning a patch test and later, when interpreting possible positive patch test results regarding clinical relevance.
- The diagnosis of allergic contact sensitisation to unidentified fragrance allergens will lead to unnecessary avoidance of other fragrance substances to which the patient is not sensitised, which are, however, included under the label “perfume”.

Furthermore, the attribute “fragrance-free” may be misleading, as it merely states that no substance was added to the product to give it a scent, assuming it is used correctly at all. Nevertheless, fragrance substances used for other purposes, e.g. as preservatives, may expose the “fragrance allergic” patient to the allergen even in a “fragrance free” product (117). However, in terms of cosmetic ingredient labelling, such other uses are less problematic, as each ingredient not used as a fragrance component must be labelled. Also the use of natural products (essential oils) as preservatives must be considered in this context.

Ingredient labelling of 26 individual fragrance ingredients, identified as allergens in humans, was introduced for cosmetics in 2005. The intention was to provide a tool for clinicians for optimizing the investigation of patients with suspected fragrance allergy, as well as for fragrance allergic patients for avoiding products containing substances they have been shown to be allergic to. Both these aims are objectives of secondary prevention and seem to have been well accepted. In a study of fragrance allergic patients and their utilisation of ingredient labelling (112), most responded that they used the ingredient labelling (86.3%) and of those who used it, the majority (65.3%) found it helpful (112). Most allergic patients used the ingredient labelling (83.2%) to find out if the product was scented, while 35.6%

also looked for specific ingredients. Many (84.9%) found that a clearer labelling, e.g. easier names and a larger font size, would increase their benefit.

4.7. Conclusions

Contact allergy to fragrances is relatively common, affecting 1 to 3% of the general population, based on limited testing with eight common fragrance allergens and about 16 % of patients patch tested for suspected allergic contact dermatitis. Fragrance contact allergy is mostly non-occupational and related to the personal use of cosmetic products.

Allergic contact dermatitis can be severe and widespread, with a significant impairment of quality of life and potential consequences for fitness for work. Thus, prevention of contact sensitisation to fragrances, both in terms of primary prevention (avoiding sensitisation) and secondary prevention (avoiding relapses of allergic contact dermatitis in those already sensitised), is an important objective of public health risk management measures.

5. Activation of weak or non-sensitising substances into sensitisers - prehapten and prohapten

Fragrance allergens act as haptens, i.e. low molecular weight chemicals that are immunogenic only when attached to a carrier protein. However, not all sensitising fragrance chemicals are directly reactive, but require previous activation.

A prehapten is a chemical that itself is non- or low-sensitising, but that is transformed into a hapten outside the skin by simple chemical transformation (air oxidation, photoactivation) and without the requirement of specific enzymatic systems.

A prohapten is a chemical that itself is non- or low-sensitising but that is transformed into a hapten in the skin (bioactivation) usually via enzyme catalysis.

It is not always possible to know whether a particular allergen that is not directly reactive acts as a prehapten or as a prohapten, or both, because air oxidation and bioactivation can often give the same product (geraniol is an example).

Some chemicals might act by all three pathways. One example is geranial (an isomer of citral) which is a hapten itself with a moderate sensitisation potency, but can be activated to more potent sensitisers via air oxidation (autooxidation) thus acting as a prehapten and also via bioactivation (metabolic activation) thus acting as a prohapten (118).

Increased understanding of the importance of activation through interaction with the environment that turns non-sensitising compounds into sensitisers has made it important to distinguish between prehapten and prohapten. This distinction facilitates discussions by emphasizing the differences in activation mechanisms between the two types of compounds requiring activation to become haptens. It is important to note that prehapten activation, in contrast to bioactivation, can be prevented to a certain extent by avoidance of air exposure during the handling and storage of the chemicals. This concerns the most prominent haptens formed by autooxidation 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 (autooxidation and metabolic oxidation) (119, 120). When haptens are formed by both pathways, the impact on the sensitisation potency depends on the degree of autooxidation in relation to the amount of metabolic oxidation.

Human data on established prehapten are presented in Table 5-1 and Table 5-2. In Table 5-1 the results from patch testing with air exposed samples of the prehapten are given. Table 5-2 shows the results from testing with the prehapten themselves without intended air exposure. In addition to the data given in this chapter, animal data (LLNA) on the pure prehapten or after controlled air exposure are given in Table 8-2. Possible pro- and prehapten are identified by SAR analyses in chapter 9.

5.1. Prehapten

Autooxidation is a free radical chain reaction in which hydrogen atom abstraction in combination with addition of oxygen forms peroxy radicals. The reaction shows selectivity for positions where stable radicals can be formed. So far, all fragrance substances that have been investigated with regard to the influence of autooxidation 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 autooxidation mixture (122). The process of photoactivation may also play a role, but further research is required to establish whether this activation route is currently underestimated in importance due to insufficient knowledge of the true haptens in this context.

Most terpenes with oxidisable allylic positions can be expected to autoxidise on air exposure due to their inherent properties. Depending on the stability of the oxidation products that are formed, a difference in the sensitisation potency of the oxidised terpenes can be seen. Oxidation products of commonly used fragrance terpenes (limonene, linalool, geraniol, linalyl acetate) have been identified as potent sensitisers in predictive animal tests (119, 123-128) (see chapter 8). This is also demonstrated for alpha-terpinene (129) and citronellol (AT Karlberg, personal communication 2012). The oxidised fragrance terpenes limonene, linalool and linalyl acetate have been tested in consecutive dermatitis patients and give frequent allergic contact reactions (130-135). Not all oxidised fragrance substances are strong sensitisers, e.g. caryophyllene is readily oxidised but has a low sensitisation potency after autoxidation (136). This is supported by clinical studies showing oxidised caryophyllene to be a less frequent allergen compared to oxidised limonene and oxidised linalool (133). Details are given in Table 5-1. The non-oxidised compounds rarely cause allergic reactions (43-45, 67, 70, 74, 97, 137-139), for details see Table 5-2. As oxidised and non-oxidised fragrance terpenes were not patch tested simultaneously in the same patients, the results are presented in two separate tables (Table 5-1 and Table 5-2).

Oxidised fragrance terpenes with defined content of the major haptens formed after autoxidation have not been commercially available for testing in dermatology clinics. In the published clinical studies testing oxidised fragrance terpenes, the patch test preparations have been obtained specifically for the performed multicentre studies. From 2012, patch test preparations of oxidised limonene and oxidised linalool with defined content of the major allergens in the oxidation mixtures, i.e. the hydroperoxides, are commercially available.

It should be noted that activation of substances via air oxidation results in various haptens that might be the same or cross-reacting with other haptens (allergens). The main allergens after air oxidation of linalool and linalyl acetate are the hydroperoxides. If linalyl acetate is chemically hydrolysed outside the skin it can thereafter be oxidised to the same haptens as seen for linalool. A corresponding example is citronellol and citronellyl acetate. In clinical studies, concomitant reactions to oxidised linalool and oxidised linalyl acetate have been observed (140, 141). Whether these reactions depend on cross-reactivity or are due to exposure to both fragrance substances cannot be elucidated as both have an allergenic effect themselves.

Linalool and linalyl acetate are the main components of lavender oil. They autoxidise on air exposure also when present in the essential oil, and form the same oxidation products found in previous studies of the pure synthetic terpenes. Experimental sensitisation studies showed that air exposure of lavender oil increased the sensitisation potency. Patch test results in dermatitis patients showed a connection between positive reactions to oxidised linalool, linalyl acetate and lavender oil (140).

Air oxidation of prehaptens can be prevented to a certain extent by measures during handling and storage of the ingredients and final products to avoid air exposure, and/or by addition of suitable antioxidants. The autoxidation rate depends not only on the compound itself, but also on its purity. The prevention of autoxidation using antioxidants needs thorough investigation because antioxidants can exert their function by being oxidised instead of the compound that they protect and might thereby be activated to skin sensitising derivatives after oxidation, which is the case for alpha-terpinene from tea tree oil (129). Alpha-Terpinene together with its analogue gamma-terpinene has been suggested as an agent for maintaining the oxidative stability of different matrices, such as food, cosmetics and medicaments (142-144). As antioxidants are now frequently used at elevated concentrations in scented products due to a growing awareness of the problem of autoxidation, there is a risk that sensitisation caused by the antioxidants will rise. One of the most used antioxidants is butylated hydroxytoluene (BHT) which is considered a minimal risk for sensitisation in the concentrations used but nevertheless, with increased concentrations and usage, the risk of sensitisation could increase.

Due to the complexity of scented products, which are mixtures of many different fragrance substances, there are at present no published data identifying the presence of individual hydroperoxides in cosmetic products containing the above fragrance terpenes. However, clinical studies show a clear connection between contact allergy to oxidised limonene and oxidised linalool, and contact allergy to other markers of fragrance contact allergy (130-135); see Table 5-3.

Table 5-1: Contact allergic reactions to the autoxidised fragrance substances limonene, linalool, caryophyllene, myrcene and linalyl acetate in consecutive dermatitis patients.

INCI name	CAS no	Test conc. (%)	n Positive/n tested (%)	Comments (Ref.)
D-Limonene (ox.)	5989-27-5	5	18/703 (2.6%)	§ (130)
		3	28/1172 (1.6%)	
		2	3/362 (0.83%)	
D-Limonene (ox.)	5989-27-5	3	63/2273 (2.8%) variation between centres: 0.3-6.5%	§ (131)
D-Limonene (ox.)	5989-27-5, 5989-54-8, 138-86-3	3	49/1812 (2.3%)	§ (134)
L-Limonene (ox.)			36/1812 (2.0%)	
D – and/or L- Limonene (ox.)			63/2411 (2.6%)	
Linalool (ox.)	78-70-6	2	20/1511 (1.3%) variation between centres: 0.4-2.7%	§ (133)
Caryophyllene (ox.)	88-44-5	3.9	2/1511 (0.1%)	
Myrcene (ox.)	123-35-3	3	1/1511 (0.1%)	
Linalool (ox.)	78-70-6	2	14/1693 (0.83%)	§ (135)
		4	67/2075 (3.2%)	
		6	91/1725 (5.3%)	
		11	72/1004 (7.2%)	
Linalool (ox.)	78-70-6	3	11/483 (2.3%)	(145)
Linalyl acetate (ox.)	115-95-7	6	13/1217 (1.1%)	(141)

Notes: § Bicentric or multicentre studies.
(ox.) Oxidised.

Table 5-2: Contact allergic reactions to limonene, linalool, linalyl acetate and caryophyllene in consecutive dermatitis patient. Please observe that several studies have been performed using the test substances without reporting the autoxidation status but it has been intended to be low. For precise information see the original references.

INCI name	CAS number	Test conc. (%)	n Positive/n tested (%)	Comments (Ref.)
Limonene	138-86-3	2	0/1200	(137)
Limonene			3/2396 (0.1%)	§ (74)
DL-Limonene			11/1241 (0.88%)	§ (43)
Limonene			0/320	(44)
DL-Limonene			3/2396 (0.1%)	§ (74)
Linalool	78-70-6	30	0/179	(139)
		20	3/1825 (0.2%)	§ (45)
		10	2/320 (0.6%)	(44)
		10	4/792 (0.5%)	(138)
		5 and 1	0/100	(70)
		10	7/2401 (0.3%)	§ (74)
Linalool, "stabilised" *	78-70-6	10	2/985 (0.2%)	§ (43)
Linalyl acetate	115-95-7	1, 5	0/100	(70)
		10	4/1855 (0.2%)	§ (67)
beta-Caryophyllene	87-44-5	5	10/1606 (0.6%)	§ (97)

Notes: § Bicentric or multicentre studies.

(ox.) Oxidised.

* Stabilised: according to the manufacturer contained additional substances aimed at limiting oxidation.

Table 5-3: Concomitant reactions to fragrance markers: Fragrance Mix I and II (FM I, FM II), *Myroxylon pereire* (MP) and to colophonium (coloph.) in the baseline series in patients with positive or negative patch test reactions to oxidised fragrance substances.

	Total number of pos. and/or neg. reactions	Pos. to FM I		Pos. to MP		Pos. to coloph.		Ref.
		n	%	n	%	n	%	
Reactions to ox. D- limonene and/or limonene hydroperoxide fraction	Pos.: 49	20	41	12	24	12	24	(130) *
	Neg.: 2751	223	8.1	142	5.2	131	4.8	
Reactions to ox. D- limonene and/or limonene hydroperoxide fraction ^a	Pos.: 60	22	37	11	18	13	22	(132) *
	Neg.: 729	141	19	71	9.7	58	8	

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Reactions to ox. D- limonene and/or ox. L- limonene ^a	Pos. to ox. D- limonene: 41	14	34	11	27	11	27	(134)*		
	Neg. to ox. D- limonene: 1771	113	6.4	91	5.1	62	3.5			
	Pos. to ox. L- limonene: 36	11	31	12	33	9	25			
	Neg. to ox. L- limonene: 1776	116	6.5	80	4.5	64	3.6			
Reactions to any of ox. linalool, myrcene, caryophyllene	Pos. to any of the tested ox. subst.: 31	12	39	6	31	12	39	(133)*		
	Neg. to any of the tested ox. subst: 1480	93	6	63	4	46	3			
		Pos. to FM I		Pos. to FM II		Pos. to MP		Pos. to coloph.		
		n	%	n	%	n	%	n	%	
Reactions to ox. linalool	Pos. at test conc. 4%: 30	8	26.7	5	16.7	10	33.3	5	16.7	(135)*
	Pos. at test conc. 6%: 55	12	21.8	8	14.5	11	20	8	14.5	
	Pos. at test conc. 11%: 72	14	19.4	9	12.5	14	19.4	9	12.5	
	Total pos. at any test conc: 75/1004	n.g.		n.g.		n.g		n.g.		
	Total neg. at any test conc: 929/1004	56	6.0	29	3.1	45	4.8	24	2.6	

Notes: * Bicentric or multicentre studies.

n.g. Not given.

(ox.) Oxidised.

5.2. Prohaptens

Compounds that are bioactivated in the skin and thereby form haptens are referred to as prohaptens. The human skin expresses enzyme systems that are able to metabolise xenobiotics (146), modifying their chemical structure to increase hydrophilicity and allow elimination from the body. Xenobiotic metabolism can be divided into two phases: phase I and phase II. Phase I transformations are known as activation or functionalisation reactions, which normally introduce or unmask hydrophilic functional groups. If the metabolites are sufficiently polar at this point they will be eliminated. However, many phase I products have to undergo subsequent phase II transformations, i.e. conjugation to make them sufficiently water soluble to be eliminated. Although the purpose of xenobiotic metabolism is detoxification, it can also convert relatively harmless compounds into reactive species. Cutaneous enzymes that catalyse phase I transformations include the cytochrome P450 mixed-function oxidase system, alcohol and aldehyde dehydrogenases, monoamine oxidases, flavin-containing monooxygenases and hydrolytic enzymes. Acyltransferases, glutathione S-transferases, UDP-glucuronosyltransferases and sulfotransferases are

examples of phase II enzymes that have been shown to be present in human skin (146). These enzymes are known to catalyse both activating and deactivating biotransformations (147), but the influence of the reactions on the allergenic activity of skin sensitisers has not been studied in detail.

Skin sensitising prohaptens can be recognised and grouped into chemical classes based on knowledge of xenobiotic bioactivation reactions, clinical observations and/or *in vivo* and *in vitro* studies of sensitisation potential and chemical reactivity. Few mechanistic investigations of prohaptens have so far been published. Investigations that are important for the bioactivation of fragrance substances are studies on alkenes, e.g. alpha- terpinene (148-150), the allylic primary alcohols geraniol (120) cinnamyl alcohol (151-155), eugenol and isoeugenol (156).

In order to be able to predict the sensitisation potency of prohaptens, steps of bioactivation have to be included in the predictive tests where intrinsic bioactivating systems are lacking. So far, no such predictive non-animal methods have been developed that take account of this.

When bioactivation occurs, the risk of cross-reactivity also needs to be considered. Cross-reactivity between certain aldehydes and their corresponding alcohols, e.g. cinnamal - cinnamyl alcohol and geranial - geraniol, due to the metabolic oxidation of the alcohols to the aldehydes in the skin is demonstrated (120, 151-155).

When using derivatives of a fragrance substance, it must be taken into account that the derivative could be metabolically transformed in the skin into the parent or cross-reacting compounds. A prominent example of such bioactivation is the hydrolysis of esters by esterases to the corresponding original alcohols. The metabolic product obtained can act as a hapten or a prohaptens in exactly the same way as the non-esterified parent compound.

Isoeugenol and its derivatives are an important example for this mechanism from which general conclusions may be drawn. As only the use of isoeugenol in fragranced products needs to be indicated on the ingredients list, the additional exposure to isoeugenol through its derivatives should also be taken into account. In a study it was shown that several EDP/EDT/aftershave lotions contained high levels of isoeugenyl acetate and isoeugenol methyl ether (Table 5-4) (157). Isoeugenyl acetate will be hydrolysed by esterases in the skin to generate isoeugenol. The situation may be similar for eugenyl acetate and geranyl acetate, which might be used in fragrance formulations instead of eugenol and geraniol, respectively. Moreover, such derivatives will contribute to exceeding any established 'acceptable dose/area level' of the parent compound, i.e., yield unduly high concentrations on the skin.

Table 5-4: Mean and median content of isoeugenol and its derivatives in the 29 perfume products.

Fragrance compound INCI Name	Products containing the fragrance		Content (ppm)			
	No.	%	Range	Mean	SD	Median
Isoeugenol	16	55	27-203	71	54	45
Isoeugenyl acetate	10	34	20-4689	985	1570	166
Isoeugenyl methyl ether	13	45	65-1755	360	442.3	222

5.3. Conclusions

- Many fragrance substances can act as prehaptenes or prohaptenes, forming allergens which are more potent than the parent substance by abiotic and/or metabolic activation. Activation can thus increase the risk of sensitisation.
- Fragrance substances of clinical importance known to be prehaptenes and to form sensitising compounds by air oxidation include limonene, linalool, and linalyl acetate.
- Fragrance substances of clinical importance known to be prohaptenes and to form sensitising compounds by metabolic transformation include cinnamyl alcohol, eugenol, isoeugenol and isoeugenol acetate.
- Fragrance substances of clinical importance with published data known to be both prehaptenes and prohaptenes and to form sensitising compounds by air oxidation (prehaptenes) and by metabolic transformation include geraniol and alpha-terpinene.
- A fragrance substance that sensitises without activation, but forms more potent sensitising compounds by air oxidation and also by metabolic transformation is, as one example, geranial (one isomer of citral).
- In the case of prehaptenes, it is possible to prevent activation outside the body to a certain extent by different measures, e.g. prevention of air exposure during handling and storage of the ingredients and the final product, and by the addition of suitable antioxidants. When antioxidants are used, care should be taken that they will not be activated themselves and thereby form new sensitisers.

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

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

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

- Further experimental and clinical research in the area of abiotic and/or metabolic activation of fragrance substances is clearly needed to increase the safety for the consumer. Compounds suspected to act as prehaptenes and/or prohaptenes should be considered as allergens, unless it could be demonstrated that they do not become activated by one of the described pathways.

6. Retrieval of evidence and classification of fragrance substances

For a systematic review, a structured approach of identifying, grading and aggregating available information should be used. Regarding the classification of substances as allergens, a number of approaches have been suggested (158-160). The categorisation of skin sensitisers according to sensitising potency has also been proposed (161, 162). For this opinion, these discussions were extended to reconcile different perspectives and to arrive at a strategy that is both consistent and applicable in practice.

By default, positive human evidence (clinical data) overrides negative results obtained in animals. This implies that the observation of a sufficient number of positive clinical cases is more important than potency information derived from animal experiments (LLNA).

Cosmetovigilance information based on consumer complaints only is of limited value in the evaluation of sensitisation risk associated with cosmetic allergens, including fragrances, as it does not identify specific causative substances, and likely to severely under-estimate the frequency of contact dermatitis. An exception is the combination with qualified diagnostic work-up, as in the French REVIDAL/GERDA system (299); however, such data are generally published, thus publicly available, and considered in the present opinion.

6.1. Retrieval of evidence

A systematic search strategy was employed for the retrieval of clinical data, as outlined below. Experimental data are often not published hence the exact definition of the scope considered for the review is necessary and is given below. Additional LLNA data were reviewed, if identified by the search strategy, e.g. in chapter 8.1.2 and, as “additional information”, in Annex I of this opinion. This supplemental evidence was, however, not considered for the final categorisation in Table 13-2.

6.1.1. Search strategy for clinical data

Method of literature search:

1. Manual search of the issues of the journal “Contact Dermatitis” (for the 26 “annex substances”, which were re-evaluated in the present opinion, starting 1999) up to October 2010, identifying all studies with fragrance substances.
2. PubMed search of CAS numbers identified in the previous opinion, reviews and already identified clinical studies, respectively, and manual screening of identified publications (narrowed for the last 10 years for the 26 “annex substances”), if necessary narrowing the search results by adding “dermatitis” or “allergy”. For example, for citral: 5392-40-5 AND (dermatitis or allergy), translated into
 "5392-40-5"[EC/RN Number] AND
 (
 ("dermatitis"[MeSH Terms] OR "dermatitis"[All Fields])
 OR
 ("hypersensitivity"[MeSH Terms] OR "hypersensitivity"[All Fields] OR "allergy"[All Fields] OR "allergy and immunology"[MeSH Terms] OR ("allergy"[All Fields] AND "immunology"[All Fields]) OR "allergy and immunology"[All Fields])
)
3. Manual search of all RIFM reviews published in supplement issues of “Food and Chemical Toxicology²” in the past 20 years. In case of the least evidence on human sensitisation the substances were preliminarily selected and further research initiated.

² Food and Chemical Toxicology, Elsevier Ltd. <http://www.sciencedirect.com/science/journal/02786915>.

4. Consideration of the most important (“top 100”) fragrance compounds in terms of volumes used (disregarding functional additives such as solvents) as supplied by the International Fragrance Association IFRA (personal communication 2010).
5. Consideration of fragrance compounds ranking 101 to 200 on the list of use volumes, if they were self-classified by manufacturers as skin sensitisers (R 43).

For the present systematic overview of available clinical data, only original studies were considered, as only these provide direct evidence, while other reviews, partly being based on the same original reports, only served to identify additional literature. In contrast, selected reviews, guidelines and similar publications were used as basis for methodological approaches (e.g., in section 11).

6.1.2. Collection of experimental (LLNA) data

The SCCS requested the International Fragrance Association (IFRA) to submit data on animal tests performed with fragrance substances, to be presented in a structured format. In response, industry submitted first a poster (163) and later a report consisting of LLNA protocol summaries on the 59 fragrance substances in the poster (164). No guinea pig studies were submitted. The SCCS has reviewed and analysed the report and the publications quoted in the report. A summary is given in chapter 8 and full data are given in Annex II. EC3 values on some additional fragrance substances in two published reviews (165, 166) have also been considered. Additional EC3 values may be available in the scientific literature and there may also be other unpublished data.

6.2. Grading of evidence

Assembled evidence has to be graded in two steps: (i) the quality of each single study, and (ii) the strength of evidence underlying the eventual classification as an allergen. Generally, studies (published or not) which are eligible for consideration will contribute to the final overall judgement to different degrees.

- Positive human data, if sufficiently demonstrated (point (i) below), will always over rule experimental (animal), *in vitro* or *in silico* data of similar internal validity, as they provide direct evidence on allergenicity in humans.
- Small study groups will contribute less precise information than larger studies of otherwise similar quality. As a minimum requirement, the size of the study groups and the numbers of events must be stated in the reports.

The following subsections will address special aspects of clinical and experimental studies, respectively.

6.2.1. Quality of a clinical study

Two major types of clinical studies must be distinguished because they provide a different scope of information:

- (i) Case reports or small case series, focusing on patients with positive (test) reactions to the target substance, sometimes including a set of non-exposed, possibly non-diseased “control patients”; these should present a concise summary of all relevant aspects of the patient's history, diagnostic procedures and possibly further outcomes.
- (ii) Clinical series in which results of a group of patients patch tested with the target substance, often combined with other substances, are presented. In the latter type of report, usually only a minority of patients tested show a positive reaction to the test substance. This implies that the majority of patients can be used to illustrate the proportion of irritant, doubtful and negative reactions. The degree of detail on the patients' histories is usually limited in such studies, compared to case reports.

Some of the basic quality criteria in clinical patch testing which should be considered are:

- Adherence to international patch test guidelines (32, 96).
- Material(s) tested should be characterised.
- Total number of patients tested must be given.
- Patient selection should be described.
- Relevance may be demonstrated either on a case-by-case basis, following pertinent guidelines, or in terms of a significant epidemiological association between sensitisation and exposure or valid markers of exposure.

Concerning relevance, it must be noted that while clinical relevance can provide important information (see 4.4.1), it is ideally based on comprehensive knowledge of prior exposures. Since the implementation of labelling 26 fragrances, previous exposure to these can often be ascertained in the assessment of relevance of a positive patch test reaction (44). However, exposure to substances not listed on a product ingredient label is obscure, except in very rare cases where elaborate diagnostics and chemical analyses are feasible (e.g. (167)). Thus, a lack of information on relevance (reported in studies) does not invalidate the impact of diagnosed contact sensitisation.

6.2.2. Quality of an experimental study

International guidelines such as the pertinent OECD guidelines for testing sensitisation have been developed and adopted. Experimental studies following these guidelines are considered as valid. However, a vast number of non-guideline studies are available and should be assessed on a case-by-case basis.

6.2.3. Quality of “other” evidence

Supporting evidence besides human and animal (experimental) data comprises *in vitro* test systems, *in chemico* experiments and structure activity relationships (SARs).

SAR analysis has at present no formal regulatory validation for skin sensitisation, nevertheless it may provide useful indicative information on sensitising potential when no or limited clinical or animal data are available.

SAR studies must consider a possible formation of haptens (allergens) from compounds able to act as prehapten by, e.g. autoxidation outside the body as well as metabolic activation in the skin of compounds able to act as prohapten (122, 168).

6.3. Aggregating evidence for a final conclusion

The criteria listed below are followed as a flow chart to arrive at a conclusion. This implies that if classification into one category is achieved, subsequent categories need not be considered. Based on the above criteria, fragrance substances were selected to be included in the present opinion if classified in one of the categories defined below.

6.3.1. Established contact allergen in humans

To qualify as an *established contact allergen*, the SCCS considers that *at least one* of the following two criteria must be met:

- At least two clinical series fulfilling the quality criteria from two different centres with cases of sensitisation, or at least three separate clinical series from different centres if a study, or studies, do not meet all quality criteria. (→ *sufficient human evidence present*)
or
- Case reports from at least two independent centres describing more than two

patients altogether in whom clinically relevant contact sensitisation had unequivocally been proven (→ *sufficient human evidence present*)
or

- At least one clinical series fulfilling the quality criteria, together with at least one case report of clinically relevant contact sensitisation (→ *sufficient human evidence present*);
or
- Experimentally induced sensitisation (e.g. unequivocally positive human maximisation tests/repeated insult patch test)³ (→ *sufficient human evidence present*).

6.3.2. Established contact allergen in animals

To qualify as an *established contact allergen*, the following criterion must be met:

- At least one positive animal study carried out according to accepted guidelines, providing evidence of a sensitisation potential (→ *sufficient animal evidence present*).

6.3.3. Likely contact allergen, if human, animal and other evidence is considered

To qualify as an *likely contact allergen*, if classification as “established ...” is not applicable, *at least two* of the following criteria must be met:

- Individual cases of allergic patch test reactions not fulfilling the requirements for sufficient evidence (→ *limited human evidence present*)
or
- At least one positive non-guideline animal study, which should be evaluated on a case-by-case basis (→ *limited animal evidence present*)
or
- Other evidence, e.g. results from *in chemico* experiments or *in vitro* tests or from structure-activity considerations based on sufficiently valid results for closely related compounds (→ *other evidence present*).

6.3.4. Possible contact allergen, if human, animal and other evidence is considered

To qualify as a *possible contact allergen*, if classification as “established ...” or as “likely ...” contact allergen is not applicable, *at least one* of the following criteria must be met:

- Individual cases of allergic patch test reactions not fulfilling the requirements for sufficient evidence (→ *limited human evidence present*)
or
- At least one positive non-guideline animal study, which should be evaluated on a case-by-case basis (→ *limited animal evidence present*)
or
- Other evidence, e.g. results from *in chemico* experiments or *in vitro* tests or from structure-activity considerations based on sufficiently valid results for closely related compounds (→ *other evidence present*).

³ It should be noted that the SCCS considers such tests unethical (169. SCCP. Opinion concerning the predictive testing of potentially cutaneous sensitising cosmetic ingredients or mixtures of ingredients adopted by the SCCNFP during the 11th plenary session of 17 February 2000. 2000:).

6.4. Conclusions

The present opinion includes (i) a well-defined search strategy for retrieving pertinent evidence; (ii) a definition of criteria used to evaluate available evidence; and, finally (iii) a set of rules to categorise the substances with regard to the relevant toxicological endpoint, i.e. sensitisation in man, based on the evidence.

7. Reported fragrance allergens from the clinical perspective

In this chapter, clinical evidence regarding sensitisation to individual fragrance chemicals and to natural extracts (essential oils) is tabulated. In this report “single chemicals” refers to chemicals of natural or synthetic origin whose chemical identity is fully known. The term “natural extracts” refers to plant or animal derived mixtures of natural chemicals, for example lavender oil, whose composition may be variable and may or may not have been fully or partly established. Full information, including possible synonyms, structural formulas (in the case of single chemicals only), a short summary of available evidence and further information, e.g. on regulatory status, is presented in Annex I.

7.1. Tabular summary of evaluated individual fragrance chemicals

Regarding nomenclature, INCI names are used wherever possible. If an INCI name is not available, the perfuming name as listed by CosIng is used. Detailed information on the publications identified and considered for this report can be found in Annex I. Several substances are currently banned from the use in cosmetic products by Annex II of the Cosmetics Directive, based on concerns regarding one or more toxicological endpoints. While available clinical evidence regarding this set of substances is listed in Annex I, these substances have not been further evaluated and are thus not included in this chapter.

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

1. Established contact allergens in humans (→ *sufficient human evidence present*).
2. Substances with positive human data, which are, however, not sufficient to categorise as “established contact allergen in humans” (→ *limited human evidence present*).
3. Substances with negative human data, i.e. patch tests of patients with suspected contact allergy to fragrance ingredients which yielded negative results.
4. Substances eligible for inclusion (see beginning of chapter 6) for which no human data are available.

A critical point in understanding this scheme is that there is publication bias in reporting allergens. This is due to the fact that once a substance has been reported and accepted as a contact allergen in humans, further reports are less likely to be published unless they are part of an epidemiological survey or when there is a novel source of exposure. Moreover, the number of patients displaying positive test reactions obviously not only depends on the underlying prevalence of sensitisation, but also on how often a substance is patch tested. This implies that inclusion of an allergen or allergen mixture in the baseline patch test series (as for Fragrance Mix I and II, *Myroxylon pereirae* and HICC, and partly also other substances/mixtures) will yield the maximum possible number of cases. In contrast, patch testing in “special” series, e.g. as a break-down of single constituents of the respective mix in case of a positive reaction to the latter, or with application only in the case of strongly suspected fragrance intolerance, will mostly result in higher relative numbers than testing the same compound consecutively, but also in lower absolute numbers.

In Table 7-1, the single substances are listed with a semi-quantification of their impact which were categorised as established contact allergens in humans according to the criteria given in chapter 6.3.

Established contact allergens in humans, according to the criteria outlined in chapter 6.3.1, were categorised according to the number of patients reacting positively and to the number of patients tested, based on the publications considered (see annex I for references). The following categories were used:

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+	Up to 10 positive test reactions reported
++	11 to 100
+++	101 to 1000
++++	> 1000

If a test allergen has been tested in less than 1,000 patients, “r.t.” (rarely tested) is added in the following tables. For this categorisation, absolute numbers of cases of sensitisation, and not the relative frequency of positive patch tests, were used, because relative frequencies depend heavily on the selection of patients for patch testing. Thereby, an important allergen tested routinely, in the baseline series, may yield 1 to 2% positive reactions (usually in several thousand patients), while an allergen tested in a selective fashion (in much fewer patients) may yield an even higher relative frequency. Moreover, case reports/series cannot be interpreted in terms of relative frequencies. The calculation of absolute numbers was based on all available literature, as detailed in the annex I to this opinion, i.e., regarding the 26 substances already listed in Annex III to the Cosmetics Directive includes data already evaluated in the previous opinion.

Table 7-1: Established contact allergens in humans (summary of evaluation as detailed in chapter 6.3). More detailed information forming the basis of this evaluation can be found in Annex I of this opinion.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment: see text
ACETYLCEDRENE	32388-55-9	+
AMYL CINNAMAL	122-40-7	++
AMYL CINNAMYL ALCOHOL	101-85-9	++
AMYL SALICYLATE	2050-08-0	+
trans-ANETHOLE	4180-23-8	+ (r.t.)
ANISYL ALCOHOL	105-13-5	+
BENZALDEHYDE	100-52-7	+
BENZYL ALCOHOL	100-51-6	++
BENZYL BENZOATE	120-51-4	++
BENZYL CINNAMATE	103-41-3	++
BENZYL SALICYLATE	118-58-1	++
BUTYLPHENYL METHYLPROPIONAL (Lilial®)	80-54-6	++
CAMPHOR	76-22-2 / 464-49-3	+ (r.t.)
beta-CARYOPHYLLENE (ox.)	87-44-5	Non-ox.: +, ox.: +
CARVONE	99-49-0 / 6485-40-1 / 2244-16-8	+ (r.t.)
CINNAMAL	104-55-2	+++
CINNAMYL ALCOHOL	104-54-1	+++
CITRAL	5392-40-5	+++
CITRONELLOL	106-22-9 / 1117-61-9 / 7540-51-4	++
COUMARIN	91-64-5	+++
(DAMASCENONE)	23696-85-7	+ (r.t.)

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment: see text
ROSE KETONE-4		
alpha-DAMASCONE (TMCHB) [#]	43052-87-5 / 23726-94-5	++
cis-beta-DAMASCONE [#]	23726-92-3	+
delta-DAMASCONE [#]	57378-68-4	+
DIMETHYLBENZYL CARBINYL ACETATE (DMBCA)	151-05-3	+
EUGENOL	97-53-0	+++
FARNESOL	4602-84-0	+++
GERANIOL	106-24-1	+++
HEXADECANOLACTONE	109-29-5	+ (r.t.)
HEXAMETHYLINDANOPYRAN	1222-05-5	++
HEXYL CINNAMAL	101-86-0	++
HYDROXYISOHEXYL 3-CYCLOHEXENE CARBOXALDEHYDE (HICC)	31906-04-4 / 51414-25-6	++++
HYDROXYCITRONELLAL	107-75-5	+++
ISOEUGENOL	97-54-1	+++
alpha-ISOMETHYL IONONE	127-51-5	++
(DL)-LIMONENE	138-86-3	++ (non-ox.); +++ (ox.)
LINALOOL	78-70-6	++ (non-ox.) +++ (ox.)
LINALYL ACETATE	115-95-7	+
MENTHOL	1490-04-6 / 89-78-1 / 2216-51-5	++
6-METHYL COUMARIN [#]	92-48-8	++ (photo-allergy)
METHYL 2-OCTYNOATE	111-12-6	++
METHYL SALICYLATE	119-36-8	+
3-METHYL-5-(2,2,3-TRIMETHYL-3-CYCLOPENTENYL)PENT-4-EN-2-OL	67801-20-1	++ (r.t.)
alpha-PINENE and beta-PINENE	80-56-8 and 127-91-3, resp.	++
PROPYLIDENE PHTHALIDE	17369-59-4	+ (r.t.)
SALICYLALDEHYDE	90-02-8	++

[#] 76/768/EEC Annex III, part 1

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment: see text
alpha-SANTALOL and beta-SANTALOL	115-71-9 and 77-42-9, resp.	++
SCLAREOL	515-03-7	+
TERPINEOL (mixture of isomers)	8000-41-7	+
alpha-TERPINEOL	10482-56-1 / 98-55-5	
Terpinolene	586-62-9	++
TETRAMETHYL ACETYLOCTAHYDRONAPHTHALENES	54464-57-2 / 54464-59-4 / 68155-66-8 / 68155-67-9	+
TRIMETHYL-BENZENEPROPANOL (Majantol)	103694-68-4	++
VANILLIN	121-33-5	++

Those substances which were categorised as +++ or more, i.e. those with the most reported cases, were also the top ranking substances in large series of patients tested with the 26 labelled fragrance ingredients ((44, 74) and additionally (170)). Geraniol is an exception, as it was all negative in the Danish study (170), but was still among the top ten in the Dutch and German studies (44, 74), with prevalences of 0.5%-0.6% positives. Geraniol has, in addition, caused many cases of contact allergy in other areas of Europe (49).

The use of absolute numbers allows the pooling of studies with different selection criteria. Limonene and linalool were not tested in their oxidized forms in the three studies (44, 74, 170) and would not have been identified, if only these publications had been the basis of assessment.

It should be noted that oxidised fragrance terpenes with defined content of the major haptens formed after autooxidation have not been commercially available for testing in dermatology clinics. In the published clinical studies testing oxidised fragrance terpenes, the patch test preparations have been obtained specifically for the performed multicentre studies. From 2012, patch test preparations of oxidised limonene and oxidised linalool with defined content of the major allergens in the oxidation mixtures, i.e. the hydroperoxides, are commercially available (see also chapter 5).

Table 7-2 lists those substances which gave rise to a few reported cases of contact sensitisation only, or where results have been reported from just one clinical department. Thus, the level of evidence concerning human data must be regarded as *limited*, according to the definitions given in chapter 6.3.

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Table 7-2: Fragrance substances with positive human data, which are, however, not sufficient to categorise as “established contact allergen in humans”. More detailed information forming the basis of this evaluation can be found in Annex I of this opinion.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment	Ref.
AMBRETTOLIDE	7779-50-2	3.4% positive reactions in 178 patients	(171)
CARVACROL	499-75-2	2 of 28 patients	(Meynadier, after (172))
CUMINALDEHYDE	122-03-2	3 of 179 patients positive	(139)
CYCLOHEXYL ACETATE	622-45-7	0.5% positive of 218 selected patients	(173)
CYCLOPENTADECANONE	502-72-7	3 of 178 patients positive	(171)
trans-trans-delta-DAMASCONE	71048-82-3	1 positive HRIPT (2/15 with 1%)	(174)
2,3-DIHYDRO-2,2,6-TRIMETHYLBENZALDEHYDE	116-26-7	1 positive HRIPT (5 of 53)	(175).
DIMETHYLTETRAHYDRO BENZALDEHYDE	68737-61-1	2.3% positive reactions isomer mixture in 178 patients	(171)
ETHYLENE DODECANEDIOATE	54982-83-1	2 / 218 positive PT reactions	(173)
ETHYL VANILLIN	121-32-4	1 occupational case	(176)
HELIOTROPINE	120-57-0	6 / 1606 consecutive patients positive	(97)
HYDROXYCITRONELLOL	107-74-4	6.0% positive PT reactions in 218 patients	(173)
ISOAMYL SALICYLATE	87-20-7	1 positive in 179 patients, possibly “excited back syndrome” 0 / 95 in another study with $\leq 1/10$ of above test conc.	(139) (70)
ISOLONGIFOLENEKETONE	33407-62-4	1 / 178 patients	(171)
METHOXYCITRONELLAL	3613-30-7	Positive PT data of unknown validity by Nakayama et al. in 22/137 patients.	(177)
METHOXYTRIMETHYLHEPTANOL	41890-92-0	0.9% positive PT	(173)
METHYL p-ANISATE	121-98-2	1 / 182 patients positive	(178)
METHYL CINNAMATE	103-26-4	6 / 142 patients positive	(179)
METHYL DIHYDROJASMONATE	24851-98-7	3 / 1606 patients positive 0 / 100	(97) (70)
METHYLIONANTHEME	55599-63-8	1 case	(180)
5-METHYL-alpha-IONONE	79-69-6	5 / 1606	(97)
METHYL OCTINE CARBONATE	111-80-8	1 case	(181)

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment	Ref.
MYRCENE	123-35-3	1 / 1511 positive to oxidized myrcene	(133)
MYRTENOL	515-00-4	2 HRIPTs with 1 pos. each	(182)
NEROL	106-25-2	6.0% positive	(173)
Nerolidol (isomer not specified)	7212-44-4	Few, unconfirmed pos. cases according to RIFM review	(183)
NOPYL ACETATE	128-51-8	2 / 179 positive, possibly "excited back syndrome"	(139)
PHENETHYL ALCOHOL	60-12-8	1 / 179; 0 / 100	(139) (70)
PHENYLACETALDEHYDE	122-78-1	1.1% of 182 positive. 1 case	(178) (184)
PHENYLPROPANOL	122-97-4	2 / 218	(173)
PHYTOL	150-86-7	1 case in human max. test	(185)
RHODINOL	6812-78-8	Several pos. HRIPTs, clinical data of uncertain validity	(186)
trans-ROSE KETONE-5	39872-57-6	2 / 22 pos. HRIPT	(187)

For a number of substances negative patch tests results were obtained, usually in rather small patient samples (max. 313 patients). For some of these substances exposure is substantial, according to data submitted from IFRA. It should be noted that a negative result does not rule out a notable sensitisation prevalence, as the study size has to be larger than, e.g. n=298 to yield a 95% CI which excludes a prevalence of 1% and larger than n=597 to exclude a prevalence of 0.5%.

Table 7-3: Fragrance substances with negative human data, i.e. patch tests of patients with suspected contact allergy to fragrance ingredients which yielded negative results.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Results / Comment	Ref.
6-ACETYL-1,1,2,4,4,7-HEXAMETHYLTETRALINE	21145-77-7	0 / 313 consecutive patients in 2 centres	(70)
AMYL CYCLOPENTANONE	4819-67-4	0 / 178	(171)
BENZYL ACETATE	140-11-4	0 / 100 consecutive patients in 1 centre observed	(70)
2-TERT-BUTYL CYCLOHEXYL ACETATE	88-41-5	0 / 313 consecutive patients in 2 centres	(70)
4-tert.-Butylcyclohexyl acetate	32210-23-4	0 / 107 consecutive patients in 1 centre observed	(70)
6-ETHYLIDENEOCTAHYDRO-5,8-METHANO-2H-BENZO-1-PYRAN	93939-86-7	0 / 178	(171)
3a,4,5,6,7,7a-HEXAHYDRO-4,7-METHANO-1H-INDEN-5(OR 6)-YL ACETATE	54830-99-8	0 / 313 consecutive patients in 2 centres	(70)
HEXYL SALICYLATE	6259-76-3	0 / 218 "top 100" substance and classified as R43	(173)
HIBISCOLIDE	6707-60-4	0 / 178	(171)
alpha-IONONE	127-41-3	0 / 205	(70)
beta-IONONE	79-77-6	0 / 205 "top 100" substance	(70)
ISOBORNYL ACETATE	125-12-2	0 / 107 "top 100" substance	(70)
METHYL ANTHRANILATE	134-20-3	0 / 91 "top 100" substance	(188)
METHYL IONONE (mixture of isomers)	1335-46-2	0 / 100 "top 100" substance	(70)
OXALIDE	1725-01-5	0 / 178	(171)
TERPINEOL ACETATE (Isomer mixture)	8007-35-0	0 / 106 "top 100" substance	(70)
alpha-TERPINYL ACETATE	80-26-2	0 / 179	(139)
TRIMETHYL-PROPYLCYCLOHEXANEPROPANOL	70788-30-6	0 / 178	(171)

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

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

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

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

7.2. Tabular summary of evaluated natural extracts/essential oils

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

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

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

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

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

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

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

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

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

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

⁴ <http://ec.europa.eu/consumers/cosmetics/cosing/>

[#] 76/768/EEC Annex III, part 1

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

Notes: r.t. Rarely tested.

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

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

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

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

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

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

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

7.3. Conclusions

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

8. Animal data

8.1. Predictive tests and sensitising potency categories

The animal test methods used in harmonised classification of substances, according to their potential to cause skin sensitisation, are the guinea pig maximisation test (GPMT), the Buehler test⁶ and the local lymph node assay (LLNA)⁷. These methods are used in hazard identification and risk assessment for regulatory purposes under REACH⁸. For registration in REACH, the LLNA is the preferred method for measuring skin sensitisation potential in animals, and justification for the use of other methods needs to be provided. According to the directives on classification and labelling⁹, substances and preparations meeting positive criteria in these tests shall be classified as sensitising and assigned the symbol “Xi” and the risk phrase “R43: May cause sensitisation by skin contact”; or, according to the recent regulation on classification, labelling and packaging (CLP¹⁰) “H317: May cause an allergic skin reaction”.

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

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

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

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

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

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

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

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

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

¹⁰ Regulation No. 1272/2008.

8.1.1. LLNA data

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

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

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

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

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

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

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

Notes: * Material with low levels of oxidation according to (164)

n/a: Not applicable (mixture of compounds).

M: EC3 based on molar concentration

8.1.2. LLNA data on oxidised fragrance substances

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

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

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

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

Notes: Pure: Purified before testing as most commercially available fragrance substances are not pure.

Not ox.: Not purified but used as it was delivered as this is a complex mixture and not a specific substance.

Ox. x w: Oxidised by air exposure during x weeks.

* Acetone:olive oil.

8.2. Methodological considerations

EC3 mean values

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

Vehicle

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

Number of doses and animals

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

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

Units for concentrations

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

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

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

8.3. Summary of animal data by LLNA

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

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

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

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

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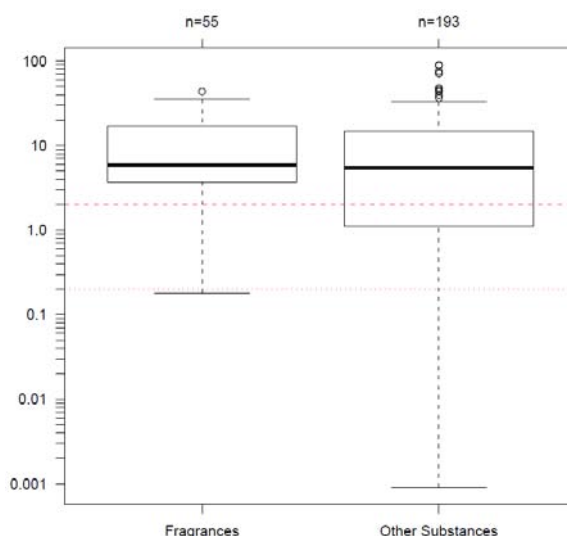


Figure 8-1: The distribution of fragrance chemicals and a variety of other chemicals (e.g. biocides, dyes, plastic materials), taken from the three references (164-166), are depicted as boxplots on a logarithmic scale. The bottom of the box denotes the 1st quartile (25% percentile), the thick line in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

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

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

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

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

8.4. Conclusions

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

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

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

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

The relationships between molecular structure and reactivity that form the basis for structural alerts are based on well established principles of mechanistic organic chemistry (214). Examples of structural alerts are aliphatic aldehydes (alerting to the possibility of sensitisation via a Schiff base reaction with protein amino groups), and α,β -unsaturated carbonyl groups, C=C-CO- (alerting to the possibility of sensitisation via Michael addition of protein thiol groups). Major mechanistic reactivity domains have been discussed in detail by Aptula and Roberts (215). Prediction of the sensitisation potential of compounds that can act via abiotic or metabolic activation (pre- or prohaptens) is more complex compared to that of compounds that act as direct haptens without any activation. The autoxidation patterns can differ due to differences in the stability of the intermediates formed, e.g. it has been shown that autoxidation of the structural isomers linalool and geraniol results in different major haptens/allergens. Moreover, the complexity of the prediction increases further for those compounds that can act both as pre- and prohaptens. In such cases, the impact on the sensitisation potency depends on the degree of abiotic activation (e.g. autoxidation) in relation to the metabolic activation.

These structural alerts can be applied by computerized expert systems, i.e. *in silico* or by estimations made by organic chemists (*in cerebro*) using their experience. When an organic chemist looks at a chemical structure, they recognise parts of the structure that they can associate with reactivity, the type of reactivity (i.e. assign the reaction mechanistic domain), and other features of the molecular structure that will affect the reactivity positively or negatively. Human experts should be aware of the complexities, and how structural modification can alter the reactivity associated with structural alerts, etc. Importantly, they can also recognise where there are unfamiliar structural features whose effects they cannot confidently predict. In such cases they can call for experimental chemistry work (*in chemico*) to be done to ascertain the presence or nature of, and degree of reactivity. *In chemico* methods include organic chemistry experimentation to identify chemical reaction products from oxidation and/or reaction with model nucleophiles, identification of mechanisms of reaction. In so called *in chemico* reactivity methods, the ability of a specific chemical to react with selected peptides is determined so as to predict the sensitisation potential of the chemical under investigation (216, 217). To make *in chemico* reactivity methods able to predict the activity of prohaptens, the addition of horseradish peroxidase and hydrogen peroxide oxidation system has been tested to model the enzymatic oxidation in the skin (218, 219).

Although computerized expert systems are derived from input by human experts, they are less well able to capture the subtleties of structure reactivity relationships, and they sometimes fail to detect aspects of chemistry that are obvious to organic chemists. Human experts should be aware of the complexities, as well as how structural modification can alter the reactivity associated with structural alerts, etc.

The SAR evaluation made in the section below is based on *in cerebro* alerts applied by organic chemists.

Depending on the type of reactivity (the **reaction mechanistic domain**), it is sometimes possible to make a quantitative prediction of potency in the LLNA, which can be used to predict potency in humans relative to related known human sensitisers. These predictions use quantitative mechanistic models (**QMMs**) based on reactivity expressed quantitatively

by model parameters, and sometimes in combination with hydrophobicity. For example, potency of aliphatic aldehydes and ketones (the Schiff base domain) in the LLNA is modelled by a combination of reactivity and hydrophobicity (220), whereas the LLNA potency of DNCB analogues (the S_NAr domain) is well modelled by reactivity alone (221).

QMMs aiming not only to predict the potential to be a sensitiser but also to predict the potency, promise to be a useful tool in non-animal based risk assessment for skin sensitisation. However, in the field of fragrance substances there are major gaps in our present ability to apply QSAR/QMM. This is largely because many of the fragrance substances of interest have the potential to act via abiotic or metabolic activation (pre- and/or prohaptens, i.e. they themselves are only weak or non-sensitisers, but have the potential to be activated to form more potent sensitisers. Resulting sensitisation potency will depend on the extent of activation and the nature of the resulting products. It is possible to apply SAR analysis to identify these plausible possibilities, but QSAR modelling for these cases is not yet developed. However, much progress has been made in identifying structural alerts for the various activation mechanisms that have been recognised. This is reviewed by Karlberg et al. (122).

Chemicals with no structural alerts for direct reactivity, or for known activation mechanisms, and no unfamiliar structural features that might be associated with as yet unidentified activation mechanisms, can be predicted to be non-sensitising. Chemicals that do have alerts for reactivity (direct or via activation) are not necessarily sensitisers – they may be insufficiently reactive and/or insufficiently hydrophobic.

Substances meeting the inclusion criteria (see chapter 6), for which, however, no categorisation as established contact allergen in humans or established contact allergen in animals was possible, have been assessed for structural alerts. The results are presented in four tables based on the prediction made for the actual substance. The following SAR assessments have been used:

- Predicted sensitiser; structural alerts:
Compounds containing structural alerts comprising direct reactive compounds and for compounds that after specific abiotic or metabolic activation (prohaptens and prehaptens) can be predicted to be sensitisers by structural comparison to known allergens.
- Possible sensitiser; structural alerts:
Compounds containing structural alerts that by comparison to known allergens with similar structures were expected to be less reactive and hence less likely to be sensitising. Also compounds with structural alerts indicating a possible abiotic or metabolic activation (possible prehaptens or prohaptens) but with no structural data available for comparison, were included in this group. Consequently, a possible sensitiser may turn out to be a non sensitiser when tested in vivo.
- Predicted non-sensitiser (NS); no obvious structural alerts
- Not predictable due to insufficient/conflicting data

Table 9-1: Predicted sensitisers.

Substance (INCI) name	CAS number	Structural alerts
p-tert.-Butyldihydrocinnamaldehyde ^s	18127-01-0	Schiff base
Citronellal	106-23-0	Schiff base and possible prehapten
Citronellyl nitrile	51566-62-2	Possible prehapten
Decanal	112-31-2	Schiff base
3,7-Dimethyl-1,6-nonadien-3-ol	10339-55-6	Prehapten
Geranyl acetate	105-87-3	Prehapten and prohapten

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Isoamyl salicylate	87-20-7	Acyltransfer agent
Methyl cinnamate	103-26-4	Michael acceptor
Methylundecanal	110-41-8	Schiff base
Myrcene	123-35-3	Prehaptent
Nerol	106-25-2	Prehaptent and prohaptent
Nerolidol (isomer not specified)	7212-44-4	Possible prehaptent
Oxacyclohexadecanone	34902-57-3	Michael acceptor
Phenethyl salicylate	87-22-9	Acyltransfer agent
trans-Rose ketone-5	39872-57-6	Michael acceptor and possible prehaptent

Note: § Classified as R43.

Table 9-2: Possible sensitisers.

Substance (INCI) name	CAS number	Structural alerts
Ambrettolide	7779-50-2	Possible prehaptent
Amylcyclopentanone	4819-67-4	Schiff base; the combination of reactivity and hydrophobicity may be enough to confer sensitisation
Benzyl acetate	140-11-4	Prohaptent via hydrolysis leading to benzyl alcohol
Carvacrol	499-75-2	Possible prehaptent
Cuminaldehyde	122-03-2	Schiff base and possible prehaptent
alpha-Cyclohexylidene benzeneacetone	10461-98-0	Possible Michael acceptor
Cyclopentadecanone	502-72-7	Schiff base; the combination of reactivity and hydrophobicity may be enough to confer sensitisation
trans-beta-Damascone	23726-91-2	Possible Michael acceptor
trans-trans-delta-Damascone	71048-82-3	Possible Michael acceptor and possible prehaptent
gamma-Damascone	35087-49-1	Possible Michael acceptor and possible prehaptent
Dihydromyrcenol	18479-58-8	Possible prehaptent
2,3-Dihydro-2,2,6-trimethylbenzaldehyde	116-26-7	Possible Michael acceptor and possible prehaptent and possible prohaptent
2,4-Dimethyl-3-cyclohexen-1-carboxaldehyde §	68039-49-6	Schiff base and possible prehaptent
Dimethyltetrahydro benzaldehyde	68737-61-1	Schiff base and possible prehaptent
6-Ethylideneoctahydro-5,8-methano-2H-benzo-1-pyran	93939-86-7	Possible prehaptent
2-Ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)-2-buten-1-ol	19-61-6	Possible prehaptent
Ethyl vanillin	121-32-4	Complex
Heliotropine	120-57-0	Possible prohaptent
3a,4,5,6,7,7a-Hexahydro-4,7-methano-1H-inden-5(or 6)-yl	54830-99-8	Possible prehaptent

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Substance (INCI) name	CAS number	Structural alerts
acetate		
Hexahydro-methanoindenyl propionate	68912-13-0	Possible prehapten
Ionone isomeric mixture	8013-90-9	Possible Michael acceptor and possible prehapten
alpha-Ionone	127-41-3	Possible Michael acceptor and possible prehapten
beta-Ionone	79-77-6	Possible Michael acceptor
Isobergamate	68683-20-5	Possible prehapten
Isolongifoleneketone	33407-62-4	Schiff base; the combination of reactivity and hydrophobicity may be enough to confer sensitisation
Longifolene [§]	475-20-7	Possible prehapten
Methoxycitronellal	3613-30-7	Schiff base
Methyl decenol	81782-77-6	Possible prehapten
Methyl ionone (mixture of isomers)	1335-46-2	Possible Michael acceptor and possible prehapten
Methylionantheme	55599-63-8	Possible Michael acceptor and possible prehapten
5-Methyl-alpha-ionone	79-69-6	Possible Michael acceptor and possible prehapten
Myrtenol	515-00-4	Possible prehapten
Nopyl acetate	128-51-8	Possible prehapten
Phytol	150-86-7	Possible prehapten and/or prohaptent
Rhodinol	6812-78-8	Possible prehapten
Terpineol acetate (isomer mixture)	8007-35-0	Possible prehapten
alpha-Terpinyl acetate	80-26-2	Possible prehapten
Tricyclodecenyl propionate	17511-60-3	Possible prehapten
Verdyl acetate	2500-83-6/ 5413-60-5	Possible prehapten

Note: [§] Classified as R43.

Table 9-3: Predicted non-sensitisers with no obvious structural alerts.

Substance (INCI) name	CAS number	Structural alerts
6-Acetyl-1,1,2,4,4,7-hexamethyltetraline	21145-77-7	
Benzyl acetone	2550-26-7	Schiff base; the combination of reactivity and hydrophobicity may not be enough to confer sensitisation
2-tert.-Butylcyclohexyl acetate	88-41-5	
4-tert.-Butylcyclohexyl acetate	32210-23-4	
Cyclohexyl acetate	622-45-7	
Diphenyl ether	101-84-8	

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Substance (INCI) name	CAS number	Structural alerts
Ethyl 2-methylbutyrate	7452-79-1	
Ethylene dodecanioate	54982-83-1	
Ethylene brassylate	105-95-3	
Eucalyptol	470-82-6	
Hexyl acetate	142-92-7	
Hibiscolide	6707-60-4	
Hydroxycitronellol	107-74-4	However, dehydration followed by autoxidation could give sensitising impurities
Isoamyl acetate	123-92-2	
Isobornyl acetate	125-12-2	
Methoxytrimethylheptanol	41890-92-0	
Methyl p-anisate	121-98-2	
Methyl anthranilate	134-20-3	
Methylbenzyl acetate	93-92-5	
Methyl dihydrojasmonate	24851-98-7	Schiff base; the combination of reactivity and hydrophobicity may not be enough to confer sensitisation
Oxalide	1725-01-5	
Pentadecalactone	106-02-5	
Phenethyl acetate	103-45-7	
Phenethyl alcohol	60-12-8	
Phenoxyethyl isobutyrate	103-60-6	
Phenylisohexanol	55066-48-3	
Phenylpropanol	122-97-4	
Tetrahydrolinalool	78-69-3	
Tetrahydro-methyl-methylpropyl)-pyran-4-ol	63500-71-0	
Trimethylhexyl acetate	58430-94-7	
Trimethyl-propylcyclohexanepropanol (tmch)	70788-30-6	
gamma-Undecalactone	104-67-6	

Table 9-4: Not predictable.

Substance (INCI) name	CAS number	Structural alerts
Anisaldehyde	123-11-5	Due to insufficient /conflicting data; structural similarities to benzaldehyde suggest certain activity in man
Trichloromethyl phenyl carbonyl acetate	90-17-5	Due to insufficient /conflicting data
Methyl beta-naphthyl ether	93-04-9	Due to insufficient /conflicting data

9.1. General results

From this work with the included SAR predictions, the following observations can be made.

- For substances for which sufficient experimental/clinical evidence is missing, SAR analyses have been performed to predict a probable or possible risk of allergenic (sensitising) effect. These predictions are based on chemical reactivity and the recognition of structural features in a substance that are in common with the structural features that have been shown to cause sensitisation from other substances. In cases where the SAR analysis indicates a sensitisation potential, the substance should be investigated further to confirm or reject the conclusion drawn from the SAR analysis.
- Prediction of the sensitisation potential of compounds that can act via abiotic or metabolic activation (pre- or prohaptens) becomes more complex compared to that of compounds that act as direct haptens without any activation.
- The complexity of the prediction increases further for those compounds that can act both as prehaptens and prohaptens.
- Prediction of the sensitisation potential of compounds that can act as prehaptens is further complicated by the fact that the autoxidation patterns can differ due to differences in the stability of the intermediates formed, e.g. it has been shown that autoxidation of the structural isomers of linalool and geraniol results in different major haptens/allergens.

9.2. Conclusions

The SAR evaluation made in this section is based on *in cerebro* alerts applied by organic chemists.

- Applying only mechanism-based QSAR (QMM) as a tool in non-animal based risk assessment for skin sensitisation is of limited value for fragrance substances. This is due to major information gaps in the present model when addressing substances that act via abiotic or metabolic activation, and the high incidence of such substances in fragrances.
- Quantitative structure activity relationship (QSAR) models should be further developed, combining, as appropriate, information from *in silico*, *in chemico* and *in vitro* methods.
- SAR, as performed here, is only one consideration in the overall weight of evidence.

10. Exposure

Exposure to fragrance chemicals and other potential allergens is most commonly by direct skin contact. Exposures to fragrance chemicals occur from:

- Personal cosmetic use;
- Detergents and other household products;
- Medicaments;
- Occupation, i.e. personal hygiene, manufacturing ingredient(s), product in work process, plant materials;
- Secondary exposure from another individual (e.g. spouse, child);
- Toys;
- Oral intake;
- Airborne exposure.

Factors that are important for both the induction and elicitation of contact allergy are:

- Dose per unit area;
- Vehicle effects including penetration enhancers;
- Presence of skin irritants;
- Presence of other allergens (combination effects);
- Duration of skin exposure;
- Frequency of applications;
- Anatomical sites of exposure;
- Condition of the skin (barrier function impairment, pre-existing inflammation);
- Occlusion (e.g. in flexures, under clothing and personal protective equipment).

Fragrance mix ingredients are commonly present in cosmetic formulations (71, 222-224). Cosmetics based on natural ingredients may contain fragrance allergens at a higher concentration than other cosmetic products (225). The clinical significance of exposure to natural extracts is difficult to determine as there is often “hidden and variable” exposure to important and potent allergens in natural products.

10.1. Concentrations and quantities used

Consumers are exposed in daily life to fragrance chemicals from a large variety of products, such as cosmetics, toys, detergents and other cleaning products, etc. The fragrance exposure may be via dermal and/or inhalation route. With respect to “Terms of Reference” to the SCCS, only dermal exposure from cosmetics is addressed in this opinion. As cosmetics are the perfumed products most commonly used in daily life, potential fragrance allergens identified by the use of cosmetics also represent the exposures of these chemicals from other product categories. In recent years, it has become a trend to add fragrance chemicals to many other types of consumer products, such as children’s toys, toilet paper and nappies, which may contribute significantly to the fragrance exposure of the consumer by the dermal route.

Factors for the fragrance exposure assessment by the dermal route require knowledge on:

- Product types (categorisation of scented products) used by the consumer.
- Market survey (impression of the qualitative and quantitative contents of different allergens in consumer products).

- Hydrolysis, metabolism or oxidation of a fragrance material, which may generate a potential skin allergen.
- Chemicals in the product matrix, which may significantly enhance or reduce dermal absorption of a fragrance material.

Fragrance materials, both defined chemical substances and natural mixtures of chemicals (essential oils), are used in all types of cosmetic products: perfumes, eau de cologne, eau de perfume (EDP), and eau de toilette (EDT), aftershave lotion, deodorants, skin care products, skin cleansers, make-up cosmetics, hair care products, and oral care products, etc. However, some unscented cosmetic products have also reached the market in the last decade. Products containing the highest concentration of fragrance chemicals are perfumes, followed by eau de cologne, eau de perfume (EDP) and eau de toilette (EDT). Concentrations of fragrance chemicals in deodorant products are lower than those in EDT/EDP products, but still significant. Aftershave products also contain relatively high amounts of fragrance chemicals. Other cosmetic products contain relatively low amounts, 0.1-1% of fragrance compound, compared to up to 30% fragrance compound in EDT/EDP (226). The fragrance compound are mixtures of 20 to over 200 synthetic fragrance chemicals or natural fragrance materials (essential oils), selected from over 3,000 fragrance materials (226). For the exposure assessment, levels of fragrance chemicals in cosmetics containing significant amounts of fragrance materials (i.e. EDP/EDT/aftershave/deodorant) should be selected. It may not be possible to detect/measure the amounts of all fragrance chemicals when present in highly diluted form in a cosmetic product such as skin care products, make-up cosmetics etc. On the other hand, if a fragrance is evaluated safe for use when present in significant amounts in a product, it will also be safe for use in other products. Also the analysis of trend of the use of individual fragrance materials should be based on monitoring their contents in fine perfumes and deodorants.

Ninety of the 100 fragrance materials used in annual volumes > 175 tons in perfume formulations are fragrances and the remaining ten are used for other functions such as solvents or antioxidants (IFRA, personal communication 2010).

Among the 26 fragrances currently requiring individual labelling, amyl cinnamal, benzyl benzoate, benzyl salicylate, butyl phenyl methyl propional, citral, citronellol, coumarin, eugenol, geraniol, hexyl cinnamal, hydroxyisohexyl 3-cyclohexene carboxyaldehyde (HICC), alpha-isomethyl ionone, and linalool are used in volumes greater than 175 ton. α -Amylcinnamyl alcohol, anisyl alcohol, benzyl alcohol, benzyl cinnamate, cinnamal, cinnamyl alcohol, farnesol, hydroxycitronellal, isoeugenol, *d*-limonene, methyl-2-octynoate, oak moss (*Evernia prunastri*), tree moss (*Evernia furfuracea*) are used in volumes less than 175 ton.

According to the information from the fragrance industry, 80% of the total fragrance chemical volume is used in cosmetics and 20% in household products.

Since the implementation of the regulation of labelling of 26 fragrance substances in cosmetic products, qualitative information on fragrance exposure from cosmetics is provided in some market surveys performed on cosmetics (Table 10-1, (227)) and (Table 10-2, (228)) and on consumer products including cosmetics (Table 10-3, (229); Table 10-4, (115); and Figure 10-1, (105)). Thus, the implementation of the regulation of fragrance allergens in detergents (Directive 648/2004/EC), similar to that for cosmetics, has also added to the knowledge of fragrance exposure to the consumer. These market surveys revealed that fragrance ingredients which are potent allergens and frequently cause allergies in consumers are used as ingredients in consumer products including cosmetics. The results of these surveys further revealed that limonene and linalool were the most commonly used fragrance chemicals in cosmetics, while anisyl alcohol, cinnamal, α -amylcinnamyl alcohol, oak moss and tree moss were the least used fragrance ingredients in cosmetics and other consumer products. In general, the most potent allergens were also the most infrequently used ingredients. Prior to the regulation of the 26 allergens, analysis of 21 selected fragrance chemicals in deodorants also revealed additional 66 potential allergens in these products on the basis of structure activity relationship (230).

Table 10-1: Presence in children's cosmetics of the 26 fragrance substances that are required to be labelled in cosmetics (227).

Fragrance substance		% Products labelled to contain the fragrance substance
INCI name	CAS number	
Amyl cinnamal	122-40-7	8.2
alpha-Amylcinnamyl alcohol	101-85-9	2.9
Anise alcohol	105-13-5	0
Benzyl alcohol	100-51-6	9.6
Benzyl benzoate	120-51-4	9.1
Benzyl cinnamate	103-41-3	2.9
Benzyl salicylate	118-58-1	9.6
Butyl phenyl methyl propional	80-54-6	7.7
Cinnamal	104-55-2	1
Cinnamyl alcohol	104-54-1	6.7
Citral	5392-40-5	8.2
Citronellol	106-22-9	10.5
Coumarin	91-64-5	4.8
Eugenol	97-53-0	7.2
Farnesol	4602-84-0	2.9
Geraniol	106-24-1	12
Hexyl cinnamal	101-86-0	10.1
Hydroxycitronellal	107-75-5	6.3
Hydroxyisohexyl-3-cyclohexene carboxyaldehyde	31906-04-4	5.8
Isoeugenol	97-54-1	0.5
Alpha-isomethyl ionone	127-51-5	5.8
<i>d</i> -Limonene	5989-27-5	23.1
Linalool	78-70-6	21.6
Methyl-2-octynoate	111-12-6	0
<i>Evernia prunastri</i> /oak moss	90028-68-5	0
<i>Evernia furfuracea</i> /tree moss	90028-67-4	0

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Table 10-2: Usage trends in deodorants of fragrance chemicals that are required to be labelled in cosmetics.

Fragrance substance		88 products investigated in 2007 (228)			70 products investigated in 1998 (231)	
INCI name	CAS number	% Products labelled to contain the fragrance	Content in 23 selected products		Content in all 70 products	
			% Products found to contain the fragrance	Range(ppm)	% Products found to contain the fragrance	Range (ppm)
Amyl cinnamal [■]	122-40-7	10.2	17	2.3-165	31	1-617
alpha-amyl cinnamyl alcohol	101-85-9	-	-	-	n.a.	n.a.
Anise alcohol	105-13-5	2.3	9	1, 51	n.a.	n.a.
Benzyl alcohol	100-51-6	17.1	26	32-166	76	1-629*
Benzyl benzoate	120-51-4	25.0	48	3-4054	71	1-1075
Benzyl cinnamate	103-41-3	3.4	9	74, 143	n.a.	n.a.
Benzyl salicylate	118-58-1	39.8	48	136-5279	49	1-18758
Butyl phenyl methyl propional	80-54-6	48.9	70	1-5455	51	1-3732
Cinnamal [■]	104-55-2	1.1	4	5	17	1-424
Cinnamyl alcohol [■]	104-54-1	12.5	48	2-503	39	6-1169
Citral [□]	5392-40-5	26.1	44	39-554	n.a.	n.a.
Citronellol [□]	106-22-9	65.9	91	1-5848	81	1-5585
Coumarin [□]	91-64-5	33.0	52	3.8-1255	57	1-1411
Eugenol [■]	97-53-0	27.3	30	1-514	57	1-2355
Farnesol [□]	4602-84-0	14.8	39	9-1791	n.a.	n.a.
Geraniol [■]	106-24-1	48.9	87	1-399	76	1-1178

Opinion on fragrance allergens in cosmetic products

Fragrance substance		88 products investigated in 2007 (228)			70 products investigated in 1998 (231)	
Hexyl cinnamal [□]	101-86-0	33.0	48	1-4434	71	2-1684
Hydroxycitronellal [■]	107-75-5	27.3	70	1-1746	50	1-1023
HICC [□]	31906-04-4	33.0	74	1-4431	53	1-1874
Isoeugenol [■]	97-54-1	9.1	35	1-138	29	1-458
Alpha-isomethyl ionone	127-51-5	46.6	65	6-2588	61	1-2765
D-Limonene [°]	5989-27-5	53.4	70	1022-11386	n.a.	n.a.
Linalool [°]	78-70-6	53.4	96	8-3447	97	9-1927
Methyl-2-octynoat [°]	111-12-6	1.1	-	-	n.a.	n.a.
<i>Evernia prunastri</i> [■] /oak moss	90028-68-5	4.6	n.a.	n.a.	n.a.	n.a.
<i>Evernia furfuracea</i> [■] /tree moss	90028-67-4	2.3	n.a.	n.a.	n.a.	n.a.

Notes: HICC Hydroxyisohexyl-3-cyclohexene carboxyaldehyde.

- Fragrance not detected in any product.

n.a. Not analysed.

* Benzyl alcohol could not be determined in 49% of the products due to interference.

The most common fragrance allergens are contained in the two mixtures, which are used for diagnosing fragrance allergy, called Fragrance Mix I (■) and Fragrance Mix II (°), besides the oxidation product of terpens (°), and tree moss extract are common allergens. Methyl-2-octynoate is an extreme, but rare allergen.

Opinion on fragrance allergens in cosmetic products

Table 10-3: Frequency of occurrence in consumer products of the 26 fragrance allergens that are required to be labelled in cosmetics and detergents (229).

INCI name of fragrance	PCP (n = 70)	MP (n = 59)	HP (n = 57)	WP (n = 44)	Cos (n = 39)	Deo (n = 17)	Dent (n = 14)	Total (n = 300)
Linalool	46	47	17	42	26	12	0	190 (63%)
Limonene	34	45	29	43	18	11	9	189 (63%)
Citronellol	23	24	21	37	25	15	0	145 (48%)
Geraniol	19	26	15	36	18	12	0	126 (42%)
BPMP	30	27	21	27	13	8	0	126 (42%)
Hexyl cinnamal	37	20	22	22	14	10	0	125 (42%)
Benzyl salicylate	23	23	10	31	15	12	0	114 (38%)
Alpha-isomethyl ionone	15	20	7	24	28	10	0	104 (35%)
Coumarin	12	27	8	23	12	8	0	90 (30%)
Lyr TM	17	24	3	24	15	5	0	88 (29%)
Eugenol	13	26	4	22	6	6	3	80 (27%)
Citral	2	28	6	29	7	2	0	74 (25%)
Benzyl benzoate	8	9	3	31	11	8	0	70 (23%)
Benzyl alcohol	9	8	1	30	9	3	1	61 (20%)
Hydroxycitronellal	5	6	1	30	6	4	0	52 (17%)
Isoeugenol	2	5	0	17	0	3	0	27 (9%)
Cinnamic alcohol	4	2	0	13	4	2	0	25 (8%)
Farnesol	1	3	0	17	2	0	0	23 (8%)
Amyl cinnamal	5	0	3	7	5	2	0	22 (7%)
Cinnamal	3	4	0	7	0	0	3	17 (6%)
Evermia prunastri/oak moss	0	3	0	5	5	0	0	13 (4%)
Benzyl cinnamate	2	0	0	8	0	0	0	10 (3%)
Evermia furfuracea/tree moss	1	5	0	3	0	0	0	9 (3%)
Anisyl alcohol	0	0	0	1	0	0	0	1 (0.3%)
Amyl cinnamic alcohol	0	0	0	0	0	0	0	0
Methyl heptine carbonate	0	0	0	0	0	0	0	0

INCI, International Nomenclature of Cosmetic Ingredients; PCP, personal care products; MP, men's products; HP, household products; WP, women's perfumes; Cos, cosmetics; Deo, deodorants; Dent, dental products; BPMP, butyl phenyl methyl propional; LyrTM, hydroxyisohexyl-3-cyclohexene carboxaldehyde.

Table 10-4: Frequency in 516 consumer products of the 26 fragrance substances that are required to be labelled in cosmetics* (115).

Fragrance substance INCI name	% Product containing the chemical
D-Limonene	48.3
Linalool	35.8
Butyl phenyl methyl propional	24.8
Geraniol	22.1
Alpha-isomethyl ionone	21.7
Hexyl cinnamal	21.3
Citronellol	21.1
Benzyl salicylate	18.6
Coumarin	17.0
Eugenol	15.7
Benzyl alcohol	15.3
Benzyl benzoate	14.7
Hydroxyisohexyl-3-cyclohexene carboxyaldehyde	12.8

Opinion on fragrance allergens in cosmetic products

Fragrance substance INCI name	% Product containing the chemical
Citral	11.6
Hydroxycitronellal	10.8
Amyl Cinnamal	7.9
Anise alcohol	7.0
Cinnamyl alcohol	6.4
Farnesol	3.9
Isoeugenol	3.1
Cinnamal	2.5
Benzyl cinnamate	2.3
Amylcinnamyl alcohol	1.9
Methyl-2-octynoate	1.0
<i>Evernia prunastri</i> /oak moss	0.8
<i>Evernia furfuracea</i> /tree moss	0.4

Note: * Consumer Products: Cosmetics and household products with labelling of the 26 fragrance allergens. The content of these fragrances was confirmed by chemical analysis.

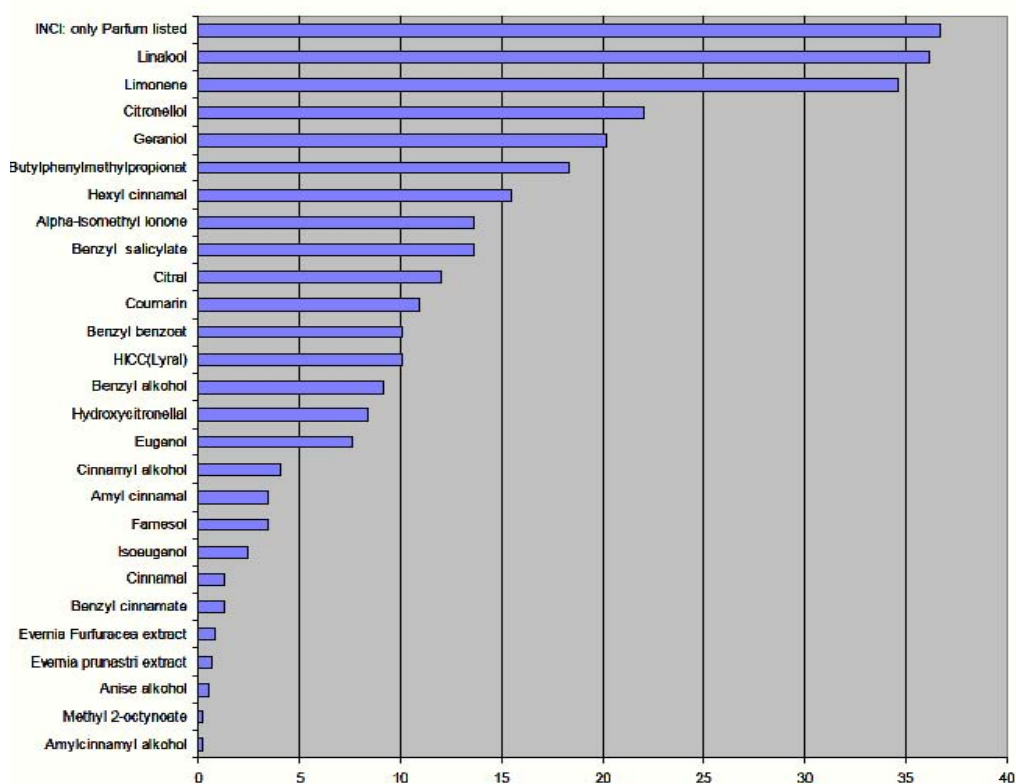


Figure 10-1: Frequency of occurrence in 3,000 consumer products of the 26 fragrance allergens that are required to be labelled in cosmetics and detergents (CVUA Karlsruhe, Germany, 2006/2007), according to (105).

Contents of fragrance substances determined in cosmetic products have been described in several studies, both before and after the regulation of the 26 fragrance allergens. The studies prior to the regulation of the 26 fragrance allergens included many, but not all of these 26 allergens. On the other hand, these studies included some other possible fragrance allergens. The quantitative analysis of fragrance substances has been performed in prestige perfumes (5, 157, 232-234), deodorants (228, 231), children's cosmetics and cosmetic toys (115, 227, 235), products marketed as natural cosmetics (225) and in cosmetics used by patients with contact allergy to fragranced products (35, 71). Quantitative analyses have revealed that the consumer is exposed to most, but not all of the 26 fragrance allergens from the use of cosmetics. However, when fragrance exposure from other consumer products, for example detergents and other household products is also taken into consideration (Table 10-3, Table 10-4, Figure 10-1), (105, 115, 229, 236), exposure to all of the 26 allergens is foreseeable in daily life. Although from the data available, the exposure to α -amylcinnamyl alcohol, cinnamal, methyl-2-octynoate, *Evernia prunastri* (oak moss) and tree moss may appear to be low, these are very strong allergens.

The changes in the use of fragrance chemicals in cosmetic formulations, during last 12 years, i.e. before and after the regulation of the 26 fragrance allergens, is reflected in the studies concerning contents of fragrances substances in popular perfumes (5, 232). As described in Table 10-5, the content of FM I allergens in prestige perfumes was significantly reduced from 1996 to 2003. Whether this is also the case for the perfumes sold as natural cosmetics (Table 10-6) has not yet been investigated.

Table 10-5: Concentration of Fragrance Mix I ingredients in five prestige perfumes before and after the regulation of the 26 fragrance allergens.

Fragrance INCI name	Concentration in the perfumes before regulation (5)			Concentration in the perfumes after regulation (232)		
	In no. of perfumes	Range % (w/w)	Mean % (w/w)	In no. of perfumes	Range % (w/w)	Mean % (w/w)
Geraniol*	5	0.072- 0.432	0.340	5	0.090- 0.236	0.156
Cinnamal	2	0.002- 0.002	0.002	0	-	-
Hydroxy- citronellal	5	0.222- 0.979	0.615	5	0.015- 0.478	0.169
Cinnamyl alcohol	4	0.068- 0.232	0.147	0	-	-
Eugenol	5	0.032- 0.738	0.337	2	0.001, 0.001	0.001
Isoeugenol	3	0.026- 0.249	0.119	2	0.001, 0.004	0.003
Amyl cinnamal	1	0.019	0.019	0	-	-

Note: * Due to interference by linalyl acetate, concentration of geraniol+linalyl acetate is reported.

Table 10-6: Concentrations of Fragrance Mix I ingredients, hexyl cinnamal and coumarin in 22 perfumes marketed as natural cosmetics investigated in 1996.

Fragrance	In no. of perfumes	Concentration % (w/w)
Geraniol	14	1.191*
Cinnamal	3	0.089, 0.109, 2.101
Hydroxycitronellal	5	0.135-6.044
Cinnamyl alcohol	8	0.035-2.289
Eugenol	2	0.027, 0.139
Isoeugenol	8	0.194-3.039
Amyl cinnamal	9	0.105-7.706
Coumarin	11	0.046-6.043

Note: * Quantification was performed in one sample only, due to interference by a very large amount of linalyl acetate in other samples.

The trend in the use of most of the fragrance allergens in deodorants before and after their regulation is reflected by the two studies performed by Rastogi et al. (228, 231). The results of these studies cannot be directly compared, because the study from 1998 included randomly selected deodorants, while selection of the deodorants for the 2007 study was based on the labelling of the presence of known strong fragrance allergens in these products. The number of products analysed in the 1998 study were three times more than those analysed in 2007, but not all of the 26 fragrance allergens were analysed in the 1997 study. However, an indication of the change in the use of the fragrance allergens during 1998-2007 may be obtained by reviewing the results of these two studies. Among the 17 common fragrance substances studied in the two studies, the frequency of use of 16 of these substances in deodorants was reduced in 2007 compared to that in 1998 (Table 10-2). The frequency of use of butyl phenyl methyl propional in deodorants appeared to be unchanged. The contents of benzyl alcohol, benzyl salicylate, cinnamal, cinnamyl alcohol, eugenol, geraniol, isoeugenol and linalool were found to be lower in the deodorants analysed in 2007 compared to those in 1998. Citronellol, coumarin and alpha-isomethylionone contents in the deodorants were similar in both studies, but concentrations of benzyl benzoate, butyl phenyl methyl propional, hexyl cinnamal, hydroxyisohexyl-3-cyclohexene carboxyaldehyde and linalool were much higher in deodorants in 2007 compared to those in 1998. This analysis of trend of use of fragrance allergens in cosmetic products indicates that the regulated fragrance allergens are used less frequently, but exposures from some of the regulated fragrance allergens may be much higher compared to those before regulation.

Table 10-7: Atranol and chloroatranol content in eau de toilette/eau de perfume, investigated in 2004 and in 2007.

	2007 Study	2004 Study
No. of samples	22	17
Atranol present in no. of samples	15 (68%)	12 (70%)
Atranol content	ppb (ng/ml)	ppb (ng/ml)
Range	n.d.-880	n.d.-791
Mean±SD	157±249	97±224
Median	47	20
Chloroatranol present in no. of samples	9 (41%)*	14 (82%)
Atranol content	ppb (ng/ml)	Ppb (ng/ml)
Range	0.9-208	1-175
Mean±SD	63±73	36±51
Median	22	10

Notes: n.d. Not detected.

* $P < 0.05$ (chi-square test).

SD: Standard deviation.

Atranol (CAS no. 526-37-4) and chloroatranol (CAS no. 57074-21-2), constituents of oak moss and tree moss have been shown to be very potent fragrance allergens (237, 238). The EC Scientific Committee on Consumer Products (SCCP) recommended that atranol and chloroatranol should not be present in cosmetic products (239). Two other commonly used fragrance chemicals, isoeugenol (240) and hydroxyisohexyl-3-cyclohexene carboxyaldehyde (HICC) (71), have also been shown to be important contact allergens. The contents of atranol, chloroatranol, isoeugenol and hydroxyisohexyl-3-cyclohexene carboxyaldehyde in fine fragrances was determined for the exposure assessment of these fragrances (233). The results revealed that isoeugenol was present in 56%, HICC in 72%, atranol in 59%, and chloroatranol in 36% of the 22 eau de toilette/eau de parfum products. The concentrations of isoeugenol were, in all products, below 0.02% which is the maximum concentration recommended by the fragrance industry. HICC reached a maximum concentration of 0.2%, which is 10-fold higher than the maximum tolerable concentration considered safe by the EC Scientific Committee (241). The concentrations of atranol and chloroatranol in the products investigated in 2007 were comparable to those found in similar products in 2004 (Table 10-7, (233, 234). A significant decrease in the frequency of the presence of chloroatranol in the products was found in 2007 (Table 10-7).

10.2. Global exposure (household and occupational exposures)

Fragrances are used in cosmetics that the consumer applies to themselves, as described in the previous section. In addition, exposure to fragrance substances is possible by a number of other exposure routes briefly outlined in this section.

Topical pharmaceutical products

In a study from Belgium, 370 of the 3,280 topical products marketed in Belgium have been found to contain one or more of 66 fragrance substances (242). This publication also contains a description of causative fragrance allergens in 127 patients reacting to 48 specific topical products. In a broader sense, exposure of the patient by extracts used in aromatherapy falls in this category as well.

Childrens products and toys

Children's products may contain fragrance allergens and high levels may be present (235). It has been stated that children may become sensitised to fragrance chemicals used by their mothers (243).

Clothing

Washed fabrics have been reported to contain fragrances (244). Odour-neutralising agents are sometimes used for shoe insoles. In one case, an insole containing cinnamon, has been reported to lead to plantar vesicular contact dermatitis due to contact sensitisation to FM I and, in the breakdown, to cinnamal and cinnamyl alcohol (245).

Cleaning agents and other household products

Contact dermatitis from geraniol in washing-up liquid has been reported (246). Terpenes are used as solvents and cleansing agents (e.g. limonene) (247) and have been reported as cause of hand dermatitis (248, 249). In an analysis of 59 household products the most common fragrance allergens were limonene (78%), linalool (61%) and citronellol (47%) (250). In a review of 301 cosmetic and detergent consumer products in Sweden, in half of the cosmetics and one-third of the detergents, one or more of the 26 fragrances requiring labelling were identified (251). In the UK, a review of 300 consumer products showed that linalool and limonene were present in 63% of products. Dental products contained on average 1.1 fragrance substances that are presently required to be labelled and women's perfumes contained 12 of these fragrance substances (Table 4-1 and Table 4-3) (229).

Candles

The dermal hand transfer of three fragrance materials (cinnamic aldehyde, d-limonene and eugenol) from scented candles was determined in ten subjects (i.e. 20 hands) after grasping scented candles for five consecutive 20 second exposures/grasps. The total mean residues of cinnamal and eugenol transferred per grasp from the candles to the hands were 0.255 µg/cm(2) and 0.279 µg/cm(2), respectively (252).

Food

Food causing cheilitis or bullous stomatitis (e.g. due to cinnamal (253)) or lichen planus-like lesions (e.g. due to cinnamal (254)) or contact gingivitis (e.g. due to eugenol (255)) has been reported. Moreover, food containing fragrance allergens, e.g. citrus oil terpenes (256) may cause allergic contact dermatitis by handling this food.

Occupational exposure

In a number of occupations, contact allergy to fragrances is more common than in others, including geriatric nurses, masseurs and physiotherapists, metal furnace operators and potters/glass makers, according to a multifactorial analysis (90). Moreover, hairdressers, beauty therapists and aroma therapists are examples of occupations where there is occupational exposure to fragrance-containing cosmetic and other products. Cleaners are exposed to fragrance-containing household products (e.g. detergents). Cooks and bakers are exposed to flavour chemicals and spices. Healthcare workers are also at risk of acquiring fragrance contact allergy. "Odour maskers" may contain important fragrance allergens (89, 90, 257-259). Occupational exposure and

occupational ACD to fragrances have been described in perfume bottlers (260). Industrial use of a powder masking the vinyl smell of car seats, containing cinnamal, causing occupational ACD has been reported (259).

A number of fragrance chemicals are also used as biocides (see Commission Regulation (EC) No 1451/2007 of 4 December 2007 on the second phase of the 10-year work programme referred to in Article 16(2) of Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market, published 11.12.2007 EN Official Journal of the European Union L 325/3 –L325/65), see Table 10-8 below.

Table 10-8: Parts of Annex I to (EC) No 1451/2007 (see above): "Active substances identified as existing", if use is 'perfuming' or 'masking' according to CosIng.

Biocide	EINECS	CAS number	Biocidal product group
Linalool	201-134-4	78-70-6	19
Geraniol	203-377-1	106-24-1	18, 19
Benzyl benzoate	204-402-9	120-51-4	2, 18
Eugenol	202-589-1	97-53-0	Not given
Farnesol	225-004-1	4602-84-0	Not given
(R)-p-mentha-1,8-diene	227-813-5	5989-27-5	12
Citriodiol/mixture of cis- and trans-p-menthane-3,8 diol	255-953-7	42822-86-6	1, 2, 19
Citral	226-394-6	5392-40-5	Not given
Pine ext.	304-455-9	94266-48-5	10
TANACETUM CINERARIIFOLIUM FLOWER EXTRACT	289-699-3	89997-63-7	18
Citrus oils (main component: limonene)	several	various	
Clove oil (main component: eugenol (83.8 %), caryophyllene (12.4 %))	/	8000-34-8	

Product groups(According to Biocide Directive 98/8/EC)

- 1 Human hygiene biocidal products
- 2 Private area and public health area disinfectants and other biocidal products
- 3 Veterinary hygiene biocidal products
- 10 Masonry preservatives
- 12 Slimicides
- 18 Insecticides, acaricides and products to control other arthropods
- 19 Repellents and attractants

The above illustrates that the consumer is exposed to fragrance substances from a wide variety of cosmetic products, other consumer products, pharmaceuticals and occupational exposures.

All these exposures are of importance in the context of contact allergy as it is not the source of exposure that is critical for both induction and elicitation, but the cumulative dose per unit area.

10.3. Exposures related to particular anatomical sites

Contact allergy to fragrances most often causes dermatitis of the hands, face and axillae. Axillary involvement has been shown to be statistically related to fragrance allergy (9). It is recognised that the axillary skin is a problematic area as it is moist, occluded and is easily irritated. Moreover, facial eczema is a common manifestation of fragrance allergy (3, 47). There is an association between fragrance allergy and hand eczema or aggravation of hand eczema (13-15). Vehicles may influence elicitation capacity of an allergen and the presence of detergents (surfactants) as in hand cleaning products may increase the clinical response by a factor of 4-6 (261). Men using wet shaving as opposed to electric razors have an increased risk of being fragrance allergic (17), most likely due to microtraumata and to the presence of surface active substances in shaving foam.

In use tests, the upper arm has been shown to be more sensitive than the forehead and lower arm (262). The axillae, neck and face are more sensitive than the upper arms (10). The threshold of elicitation may vary depending on the volatility of the substance (263). A cumulative effect of exposures occurs so that repeating exposures cause elicitation in more individuals (264).

Patients appear to become sensitised to fragrances primarily from deodorants and perfumes and to a lesser extent from other cosmetic types (74). Allergic contact dermatitis may develop where a perfume has been applied (behind ears, neck, upper chest, antecubital fossae, wrists and the axillae bilaterally (265). Following this, eczema may appear, or be worsened by, the use of a variety of product types including other cosmetics, household products, industrial products and flavours.

The association between contact allergy to fragrance ingredients and certain anatomical sites, which mirrors exposure to fragrance-containing products on these anatomical sites, has been described in several publications (266, 267), see above. However, due to the potential confounding effect of other factors, at least on some anatomical sites, an adjusted analysis will provide a more valid impression of the association between certain anatomical sites and contact allergy to fragrance ingredients. As an adjusted, multifactorial analysis relies on: (i) a substantial number of observations (patients tested); and (ii) an outcome prevalence not too close to 0%, such an approach has, hitherto, been limited to FM I.

In a paper published 2001, data from the IVDK in terms of patch test reactions to FM I and relevant clinical and demographic information of the patients tested (n=57,779) was studied by Poisson regression analysis (90). Risk was quantified by the prevalence ratio, which can be interpreted as an estimate of relative risk, i.e. the factor by which the risk of being sensitised to FM I (in this example) is to be multiplied (RR > 1: elevated risk; or RR < 1: reduced risk) if a certain "risk factor" is present, compared to those patients in whom this risk factor is not present (the reference category) (general aspects of such analyses are discussed in (268)). In the analysis, potential risk factors and confounders, respectively, including occupation, year of patch testing (to address a possible time trend), sex, age, past or current atopic dermatitis, in addition to anatomical site. The relevant part of Table 3 of (90) is reproduced below.

Table 10-9: Result of a Poisson regression analysis of patients tested with the Fragrance Mix between January 1992 and December 1998, considering two alternative outcomes – part I: non-occupational factors

Attribute	Prevalence (%)	At least + (11.5%)		At least ++ (4.0%)	
		PR	95% CI	PR	95% CI
Age:					
≤30	26.7	1.00	Reference	1.00	Reference
>30–44	23.8	1.42	1.31 to 1.53	1.61	1.40 to 1.84
>44–58	25.6	1.67	1.55 to 1.80	1.90	1.66 to 2.16
>58	23.9	1.93	1.77 to 2.10	2.07	1.79 to 2.39
Sex (female)	64.5	1.29	1.21 to 1.37	1.18	1.07 to 1.31
Main site:*					
Trunk	2.9	1.00	Reference	1.00	Reference
Hands	29.9	1.24	1.07 to 1.46	1.28	0.98 to 1.67
Arm	3.8	1.23	1.01 to 1.49	1.19	0.86 to 1.65
Face	15.2	1.20	1.03 to 1.42	1.13	0.86 to 1.48
Neck	1.4	1.39	1.10 to 1.75	1.31	0.88 to 1.94
Feet	2.8	1.26	1.02 to 1.55	1.19	0.84 to 1.68
Leg	8.7	1.59	1.36 to 1.89	1.50	1.14 to 1.99
Axilla	0.9	2.77	2.20 to 3.46	2.73	1.87 to 4.00
Other site	8.9	0.66	0.55 to 0.80	0.48	0.35 to 0.67

*Additionally controlled for several more sites—none of these associated with a significantly increased or decreased risk.

Compared to the trunk, which was arbitrarily chosen as the reference category, all other anatomical sites are associated with an increased risk of being sensitised to FM I (significantly if the lower limit of 95% CI is > 1). Most evidently, dermatitis of the axilla(e) is strongly associated with contact allergy to FM I, presumably due to the application of deodorants. Furthermore, the part of the table shown above illustrates a strong, positive age gradient, i.e. the older patients are, the more likely they are to be sensitised to FM I, the risk being almost double when comparing the oldest with the youngest age group. This observation is in concordance with a bivariate (unadjusted) association between age and contact allergy to FM I found in another study (89). This association is presumably the result of life long exposures and cumulative risk.

In a similar analysis of *Myroxylon pereirae* resin, published in 2002 (269): (i) an even stronger age gradient; and (ii) no particular association to axillary dermatitis (included in the “other” category) was found (Table 10-10).

Table 10-10: Association between selected risk factors and positive patch test to *Myroxylon pereirae* resin. For full model see (269). Risk quantified with the prevalence ratio (PR) with accompanying 95% confidence interval (CI).

Factor	PR	95% CI
Atopic dermatitis, past or present	1.02	(0.95-1.10)
Female sex	1.13	(1.06-1.20)
Site		
Trunk	1.00	(reference)
Hand or Arm	1.03	(0.94-1.12)
Foot or Leg	1.76	(1.61-1.92)
Head or Neck	0.94	(0.86-1.03)
“Other” site	0.72	(0.64-0.81)
Missing site	1.07	(0.97-1.19)
Age		
30 years and younger	1.00	(reference)
31 to 44	1.92	(1.73-2.12)
45 to 58	2.87	(2.61-3.16)
58 or older	3.85	(3.49-4.25)

10.4. Conclusion

There are various modes of exposure to fragrances, including not only products used for their scent, such as perfumes and eau de toilette, after shaves, and deodorants, but also types of products where scent is an added feature, such as other cosmetic categories (including wipes), topical pharmaceuticals, household products, and products encountered in the occupational setting.

Consumer exposure can change over time, both qualitatively and quantitatively.

Different routes of exposure are reflected by certain anatomical sites affected: deodorants are associated with axillary dermatitis, the axillary skin being particularly vulnerable to sensitisation due to occlusion, maceration and irritation. However, while sensitisation and initial disease may follow a distinct pattern, later less specific exposures, e.g. via hand creams, cleaning lotions etc. may be sufficient to cause allergic contact dermatitis.

11. Dose-response relationships and thresholds

The dose-response relationship between exposure to contact allergens and induction of allergy, i.e. sensitisation, is well established in animal models and by experiments in healthy volunteers (270). It seems that not only the dose per unit area of allergen (271), but also the number of exposures, i.e. the accumulated dose, is of importance for the risk of induction of contact allergy (272). The induction of contact allergy is an immunological process (type IV-allergy), which is without any clinical symptoms. In the case of continued exposure or re-exposure with a sufficient dose of allergen, elicitation will occur. Elicitation is an inflammatory response (eczema) with clinical symptoms of erythema, induration and in some cases vesicles. Studies of the elicitation response are normally done in patients with an allergy to the substance in question. Different provocation models exist (see chapter 11.2.1). Elicitation experiments in healthy human volunteers following the induction have only rarely been performed (273) and may be considered a less valid model than patient studies. The reason is that following experimental induction, the level of sensitivity may not be at the same level as in a real life situation and that individuals who have actually acquired the disease are a more relevant endpoint to study.

Knowledge of the dose-response relationship provides an opportunity to establish levels of exposure which are safe for the majority of individuals. In the following chapter, the use of different data and models for the establishment of such safe levels in relation to fragrance ingredients are explored. The focus will be on those chemicals, which have been identified in chapter 7.1 as established contact allergens in humans and which have already given rise to a significant number of published cases (category 3 or more): cinnamal, cinnamyl alcohol, citral, coumarin, eugenol, farnesol, geraniol, hydroxycitronellal, isoeugenol. Limonene and linalool are considered in chapter 5 as their ability to cause sensitisation depends on air oxidation, and hydroxyisohexyl 3-cyclohexene carboxaldehyde is considered in chapter 4.3.2 and 11.4.

11.1. Induction

A model for dermal sensitisation quantitative risk assessment (QRA) has been developed and implemented by the fragrance industry. This model relies on thresholds, no effect or low-effect levels, established in healthy human volunteers and/or in animal experiments, mainly the local lymph node assay (LLNA) (see chapter 8.1). A set of safety factors are applied for inter-individual differences, for vehicle effects and for use considerations, stated to give rise to a safety margin from 10 to 1000 (274). In this way, a so-called “acceptable exposure level” is derived. The exposure to an allergen in different types of products should be below this level. The restrictions, which have been introduced by the fragrance industry based on the QRA model, are given in Table 11-1 for some important product categories.

The IFRA guidelines give concentration limits for 11 product categories (http://www.ifraorg.org/en-us/standards_1, last accessed 2011-11-02), three of which are mentioned in Table 11-1. These three products have the lowest concentrations except for lip products, which give a slightly lower concentration limit.

Table 11-1: Current IFRA restrictions based on induction experiments.

Fragrance chemicals	IFRA guideline ¹		
	Deodorant (%)	Hand cream (%)	Perfume (%)
Cinnamal	0.02	0.05	0.05
Cinnamyl alcohol	0.1	0.4	0.4
Citral	0.05	0.3	0.6
Coumarin	0.13	0.8	1.6
Eugenol	0.2	0.5	0.5
Farnesol	0.11	0.6	1.2
Geraniol	0.4	2.8	5.3
Hydroxycitronellal ²	0.2	1.0	1.0
Isoeugenol ²	0.01	0.02	0.02

Notes: 1) Exposure per mg/cm²/day is based on 8.5 mg/cm²/day for deodorants, 2.2 for perfumes and 4.2 for hand creams as it is these exposure levels that are used by the IFRA.
 2) Cosmetic Directive Annex III: Hydroxycitronellal restricted to 1% in all products and isoeugenol to 0.02% in all products.

The SCCP evaluated this methodology (275) as well as its application to three model fragrance substances.

It was, among other things, concluded that:

“The data provided show that the application of the dermal sensitisation QRA approach would allow increased exposures to allergens already known to cause allergic contact dermatitis in consumers. The model has not been validated and no strategy of validation has been suggested. There is no confidence that the levels of skin sensitisers identified by the dermal sensitisation QRA are safe for the consumer.”

and that:

“Identification of safe levels of exposure to existing substances known to cause allergic contact dermatitis in the consumer should be based on clinical data and/or elicitation low-effect levels. Currently, these are the only methods which have proven efficient in reducing/preventing existing problems of sensitisation/allergic contact dermatitis in the consumer.”

11.2. Elicitation

11.2.1. General considerations

A response in terms of elicitation of allergic contact dermatitis by application of the (suspected) allergen under standardised conditions is the outcome of interest of the routine diagnostic procedure for suspected contact allergy, the patch test. While the patch test procedure is largely standardised, it is optimised as a diagnostic tool for contact allergy. Thus exposure conditions are not comparable to actual exposures occurring in the daily life or working environment of the patient, which often involve long-term, repeated and low-dose contact with the allergen. Here, procedures such as the repeated open application test (ROAT) or provocative use test are often used, because they reflect actual exposure much better and can be used, for instance, to validate the current clinical relevance of a positive PT reaction.

Generally, exposure of a sensitised patient to a set of graded doses (quantity/area) of the suspected allergen, i.e. threshold testing, will allow not only quantitative diagnosis of the presence or absence of specific contact sensitisation but will additionally provide evidence on the intensity (degree) of sensitisation. This may have important individual consequences in terms of everyday or occupational exposures being capable (or not) of eliciting allergic contact dermatitis. However, beyond the individual perspective, clinical dose-response data collected from sensitised individuals provide a valuable estimate of the usual doses/unit area resulting in a positive, allergic response in a certain proportion of sensitised persons, e.g. 10, 50 or 90%. Maximum concentration levels can be derived, which are safe in terms of eliciting allergic reactions in only a defined low percentage of sensitised persons. As such data will always be based on small samples, the precision of the estimate should be considered, and therefore results are preferably given with confidence intervals.

A statistically significant relationship between threshold concentrations in the ROAT and patch test has been found, on analysing results from different allergens (see Table 11-2) (276), but the dose of allergen per unit area per application needed to elicit a reaction in the two study methods is not the same. A translation factor between the two methods has been suggested for non-volatile substances: $ED_{xx}(ROAT) = 0.0296 \cdot ED_{xx}(\text{patch test})$ based on testing nickel and methyl dibromo glutaronitrile (276). Based on this the eliciting dose per application in an open test is 33 times lower than in the patch test. In practice it means that the cumulative dose in a ROAT (in $\mu\text{g}/\text{cm}^2$) in two weeks with two applications per day (total 28 applications) will be almost identical to the eliciting patch test dose (in $\mu\text{g}/\text{cm}^2$) for a given number of responders (see Figure 11-1). For a given cut-off point the elicitation dose determined by patch testing will be higher than determined by ROATs.

Table 11-2: Spearman's rank correlation between the threshold concentration in the patch test and the repeated open application test for three allergens.

Allergen	Number of patients	Correlation coefficient	P-value
Nickel	18	0.45	0.033
MDBGN	15	0.76	0.0021
HICC	16	0.59	0.011

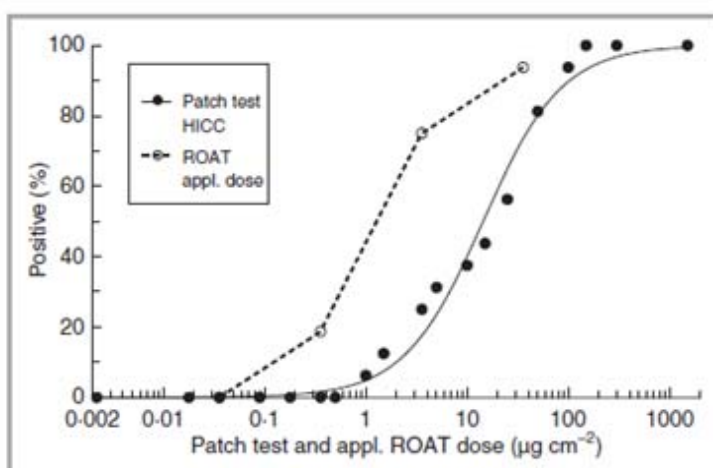


Figure 11-1: The fitted dose-response curve for patch test (solid line) is seen to be displaced to the right compared to the observed response from repeated open applications of the same allergen (HICC). It means that a smaller dose per application is needed to elicit a response than by one single occluded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

In the translation between methods, evaporation needs to be taken into consideration for volatile substances. The experience, based on a study of the fragrance ingredient HICC and using the results from the literature on isoeugenol, is that if the same equation is used as for non-volatile substances, the response in the ROAT will be overestimated by a factor 3 to 4. Thus, the translation factor would be 0.1060 instead of 0.0296, but this needs to be confirmed by other fragrance allergens. This implies that for the fragrance ingredients tested, the eliciting dose per application in a ROAT was 9.4 times lower than the patch test compared to a 33 times lower dose for non-volatile substances (276). This needs to be confirmed by studying other fragrance allergens. Thus, according to these experiments, the dose ($\mu\text{g}/\text{cm}^2$) eliciting a response in threshold patch testing will be at most 33 times higher than established in the ROAT if an identical vehicle is used.

Volatility effects in skin sensitisation

The potency of volatile skin sensitisers can be underestimated, to an extent depending on how rapidly it evaporates, by assays such as the LLNA in which the test substance is applied topically to exposed healthy skin without occlusion. Such sensitisers present a greater sensitisation risk to consumers when the skin is occluded by clothing and/or compromised, than when healthy non-occluded skin is exposed.

Volatility at physiological temperature, say 40°C , is represented by the vapour pressure p_{40} at that temperature. This is related to the boiling point T_B by the Clapeyron-Clausius equation, which can be written (277):

$$\text{Log}(p_{40}) = - (T_B - 40)\text{Tr}/2.303RT$$

Where p is in atmospheres, T_B is in $^\circ\text{C}$, R is the gas constant, Tr is the Trouton constant (also defined as the molar entropy of vaporisation, and equal to $22 \text{ cal}\cdot\text{deg}^{-1}$ for many organic compounds) and T is physiological temperature in degrees absolute ($= 313$ for 40°C).

It has been shown, in experiments where evaporation from a glass slide is measured under simulated LLNA conditions, that 2-hexenal ($T_B = 146\text{-}149^\circ\text{C}$, $p_{40} = 17 \text{ mmHg}$) evaporates rapidly, less than 20% remaining after 5 minutes, whereas with cinnamal ($T_B = 248^\circ\text{C}$, $p_{40} = 0.5 \text{ mmHg}$), more than 90% remains after 1 hour (278). In agreement with these findings, cinnamal fits a QSAR relating LLNA EC3 to reactivity, whereas the EC3 for 2-hexenal is higher (lower potency) than predicted from its reactivity.

The above is only a partial rationalisation, since different solubilities in different vehicles will influence the tendency to evaporate, according to Henry's law.

11.2.2. Studies on specific fragrance ingredients

Studies concerning chloroatranol/atranol, cinnamal, hydroxycitronellal, hydroxyisohexyl 3-cyclohexenecarboxaldehyde and isoeugenol have been identified. These are summarised in Annex III.

Overview of results

In four studies dummy deodorants spiked with a single fragrance allergen in realistic use concentrations have been used to study elicitation responses, unscented deodorants were used as control products in paired designs. The deodorants were used by patients sensitised to the fragrance allergen in question as well as a healthy control group

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(without fragrance allergy) (102,103,104,279). Between 76 and 100% of the sensitised individuals reacted to the deodorants spiked with allergen, isoeugenol, cinnamal, hydroxycitronellal and hydroxyisohexyl 3-cyclohexene carboxaldehyde, and none of the controls (Table 11-4).

Table 11-3: Overview of results of deodorant provocation investigations with different allergens. Frequency in % of test groups, which reacted at different doses of allergen applied in a roll-on deodorant in the axilla, is given in the table.

Dose in ppm in deodorant	Isoeugenol	Cinnamal (1)	Cinnamal (2)	Hydroxycitronellal	HICC
0	0	0	0	0	0
63	23				
100			11		
200	69				64
320		25	55	57	
600					85
630	76				
1000		75	88	71	
1800					100
3200		100		100	
No. test persons	13	8	9	7	14
No. of control persons	10	20		7	10
% control persons, who reacted	0	0		0	0
Exposure according to study should be:	< 63 ppm	<100 ppm		<320 ppm	< 200 ppm
Reference	(279)	(103)		(104)	(102)

Note: HICC hydroxyisohexyl 3-cyclohexene carboxaldehyde.

Eleven studies concerning dose-response results of the five allergens listed above were identified, including the above mentioned studies of deodorants. An overview of the results of the studies concerning thresholds is given in Table 11-4. In Annex III the details of each study are given.

Table 11-4: Overview of threshold results from clinical studies.

“Observed” means that the proportion was actually observed in the study while “estimated” means that the value is derived from a fitted curve, i.e. is interpolated.

Chloroatranol			
ROAT			Ref.
In ethanol 92 % positive	0.025 µg/cm ²	observed	(238)
In ethanol 100% positive	0.125 µg/cm ²	observed	(238)
PATCH TEST			

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ED10%	0.0004 µg/cm ²	estimated	(238)
ED50%	0.0045 µg/cm ²	estimated	(238)
Cinnamal			
ROAT			
In ethanol no effect	0.02%	observed	(101)
In ethanol 44 % positive	0.1%	observed	(101)
In ethanol 72 % positive	0.8%	observed	(101)
Deodorant matrix 11% positive	0.26 µg/cm ² (0.01%)	observed	(103)
Deodorant matrix 41% positive	0.84 µg/cm ² (0.032%)	observed	(103)
Deodorant matrix 82% positive	2.63 µg/cm ² (0.1%)	observed	(103)
PATCH TEST			
ED50%	96 µg/cm ²	estimated	(101)
No effect level	0.4 µg/cm ² (0.01%)	observed	(101)
No effect level	NG (0.002%)	observed	(103)
HICC			
ROAT			
In a cream base ED10%	4.9 µg/cm ²	interpolated	(105)
In a perfume (ethanol) ED10%	1.2 µg/cm ²	interpolated	(105)
In ethanol 61% positive	15.3 µg/cm ² (3.4-22.2)	observed	(224)
In ethanol 89% positive	126.2 µg/cm ² (40.5-226.2)	observed	(224)
In ethanol/water no response	0.0357 µg/cm ²	observed	(263)
In ethanol/water ED10%	0.064 µg/cm ²	estimated	(263)
In deodorant matrix between 64% to 100% positive	0.79 µg/cm ² (median)	observed	(102)
PATCH TEST			
ED10% (95% CI)	0.662 µg/cm ² (0.052-2.35)	estimated	(263)
ED10%	0.75 µg/cm ²	estimated	(102)
ED10%	0.9 µg/cm ² 29 (7-69) ppm	estimated	(224)
ED50% (95% CI)	11.1 µg/cm ² (3.41- 33.1)	estimated	(263)
ED50% (95% CI)	18.3 µg/cm ² (3.41- 33.1)	estimated	(102)
ED50% (95% CI)	20 µg/cm ² 662 (350-1250) ppm	estimated	(224)
No effect level	<0.0022 µg/cm ²	observed	(263)
Hydroxycitronellal			
ROAT			
Deodorant matrix 57 % positive	0.94 µg/cm ² (0.032%)	observed	(104)
Deodorant matrix 71 % positive	2.94 µg/cm ² (0.1%)	observed	(104)
Deodorant matrix 100 % positive	9.40 µg/cm ² (0.32%)	observed	(104)
PATCH TEST			

No effect level	<0.00012 % (=0.036 µg/cm ²)* (*calculated)	observed	(104)
Isoeugenol			
ROAT			
in ethanol 63% positive	5.6 µg/cm ²	observed	(100)
in ethanol 42% positive	2.2 µg/cm ²	observed	(264)
in ethanol 67% positive	9.0 µg/cm ²	observed	(264)
Deodorant matrix 23 % positive	0.167 µg/cm ²	observed	(279)
Deodorant matrix 69 % positive	0.53 µg/cm ²	observed	(279)
Deodorant matrix 77 % positive	1.67 µg/cm ²	observed	(279)
PATCH TEST			
ED50% (in petrolatum)	32 µg/cm ²	estimated	(100)
No effect (in ethanol)	<0.0005% (0.15 µg/cm ²)	observed	(264)
No effect (in petrolatum)	<0.4 µg/cm ²	observed	(100)

Summary of results for specific fragrance ingredients

Chloroatranol (constituent of *Evernia prunastri*)

In ROAT a dose of 0.025 µg/cm² to 0.125 µg/cm² in ethanol elicited reactions in 92% to 100% of sensitised subjects.

In patch testing the ED10% was 0.0004 µg/cm².

Cinnamal

In ROAT a dose of 0.26 µg/cm² gave a response in 11% when applied as deodorant in the axilla and 82% responded to 2.63 µg/cm².

The ED50 in patch testing was 96 µg/cm².

HICC

In ROAT a dose of 0.0357 µg/cm² gave no response, while the dose that elicited a reaction in 10% of the sensitised test group (in ethanol) ranged from 0.064 µg/cm² to 1.2 µg/cm². The dose in a cream base was 4.9 µg/cm².

In ROAT a dose of 15.3 µg/cm² to 126.2 µg/cm² in ethanol elicited reactions in 61% to 89% of sensitised subjects.

The ED10 in patch testing ranged from 0.66-0.9 µg/cm².

Hydroxycitronellal

In ROAT a dose of 0.94 µg/cm² gave a response in 57% when applied in a deodorant in the axilla and 100% responded to 9.40 µg/cm².

The no-effect level in patch testing was below 0.036 µg/cm².

Isoeugenol

In ROAT a dose of 2.2 µg/cm² a response in 42% and 9.0 µg/cm² in 67%, when applied in ethanol on the arm. With a deodorant applied to the skin of the axillary, a dose of 0.167 µg/cm² caused a response in 23% and 77% reacted to 1.67 µg/cm².

The ED50 in patch testing was 32 µg/cm².

The no-effect in patch testing was below 0.15 µg/cm².

Elicitation levels have been studied for cinnamal, isoeugenol and hydroxycitronellal which are established contact allergens in humans and which already have given rise to a significant number of cases (> 100, see chapter 7). Further HICC has been studied extensively, but is considered in a separate section (chapter 11.3) of this opinion. It is however not possible to derive a safe threshold directly from the data of cinnamal, isoeugenol and hydroxycitronellal. The main reasons are that many of the test subjects reacted to all the tested doses in ROAT, which is a simulation of every day exposures. Thus it was not possible to determine the dose only eliciting responses in a few, e.g. 10% of the subjects and that only a limited number of exposure scenarios were studied.

The studies have covered few product types: hydro-alcoholic products, e.g. perfumes and deodorant roll-on matrix. The vehicle is one of many factors which influence the thresholds of allergic reactions. Also the presence of irritants and other allergens can influence the elicitation level. This means that the currently available studies do not cover all the relevant exposure scenarios. However, taking into account that dose-response investigations in sensitised patients are very complex to perform, it is not likely that much more data will become available in the near future. It is therefore necessary to exploit the full pool of elicitation data, also covering chemicals other than fragrance ingredients, to derive a more general threshold which could be used when no or insufficient data exist to set a specific threshold for a substance of concern.

General thresholds

The methodology of the different experiments has varied to some extent as different anatomical sites of exposure have been employed, different vehicles, exposure periods and cut-off points. The reason is that the studies have been performed to investigate various clinical and scientific aspects of allergic contact reactions and not for formal regulatory requirements. Some studies are small and for this reason the precision of the estimates of thresholds is limited. In spite of this, the results of the various experiments are reasonably uniform, except for chloroatranol which had very low threshold reactions, and show that low concentrations may elicit allergic reactions.

The reasonably uniform data generated on the above fragrance ingredients are in agreement with a recent “meta-analysis” of dose-response data of different allergens, incorporating some of the same studies as mentioned above, but also other allergens, such as preservatives and metals. The ED₁₀ at patch testing varied by a factor of 7 from the lowest to the highest value and the median was 0.82 µg/cm² if the three outliers formaldehyde (1997), nickel (1999) and methyldibromo glutaronitrile (2004) were left out and 0.84 µg/cm² if included (see Table 11-6 and Figure 11-2 below: (280)). An explanation of these results could be that thresholds in elicitation is less dependent on the antigenic properties of the individual substance (inherent potency) than thresholds of induction and more on the level of sensitivity of the individual, i.e. the level of T-cell clones able to recognise the antigen, which is not present in naïve not-sensitised, individuals. This seems plausible, based on both the recent clinical evidence (280) and guinea pig QSAR evidence (281). It provides the basis for a general approach in establishing safe thresholds for substances of concern.

The consequences of a limit of 0.8 µg/cm² for the product types most important for fragrance allergy are calculated below.

The calculation is based on:

- The generally safe exposure level, which is the median ED₁₀ value (the dose which will elicit allergic contact dermatitis in 10% of sensitised eczema patients) under patch test conditions: 0.8 µg/cm² (280).

- Exposure doses and exposure areas from SCCS notes of guidance 7th revision (282) [Tables 2 and 3] and Technical dossier Quantitative Risk Assessment from RIFM (274).

Equation:

Safe concentration in product = (Generally safe exposure level ($0.8 \mu\text{g}/\text{cm}^2$)/daily exposure to product ($\mu\text{g}/\text{cm}^2/\text{day}$)) x 100 (for %).

Table 11-5: Concentration limits in different product types based on $0.8 \mu\text{g}/\text{cm}^2$ allergen as a 'generally safe exposure level', if specific dose-response data are unavailable.

	Estimated daily exposure level (g) (Table 3 SCCS NoG)	Mean exposed skin surface (cm^2) (Table 2 SCCS NoG)	Exposure $/\text{cm}^2/\text{day}$ in grams	Exposure $/\text{cm}^2/\text{day}$ in μg ($1\text{g} = 1 \times 10^6 \mu\text{g}$)	Concentration limit in product % in product: (GEL/daily exposure) x 100
Body lotion	7.82 g	15,670 cm^2	0.000499	499	0.16%
Face cream	1.54 g	565 cm^2	0.002725	2725	0.03%
Hand cream	2.16 g	860	0.002511	2511	0.03%
Deodorant aerosol spray ethanol based	1.43 g	200 cm^2	0.007150	7150	0.01%
Perfume spray	not given	?	0.00221 ¹⁾	2210	0.04%

Note: 1) $2.21 \text{ mg}/\text{cm}^2/\text{day}$ from Technical dossier Quantitative Risk Assessment.

The estimated daily use of the various product categories in Table 11-5 are based on the SCCS Notes of Guidance (see above), except for perfume, for which no value is given. This value is taken from the Technical Dossier on Quantitative Risk Assessment from RIFM.

Generally the estimated use of different products is higher in the IFRA/RIFM assessments than in SCCS Notes of Guidance.

Table 11-6: Overview of dose-response studies and thresholds for eight allergens, after (280).

ED₁₀ patch test values from each of the 16 selected studies with 95 % confidence intervals with the allergens chromium (283), MCI/MI (Kathon TM CG) (284), nickel (285), methylidibromo glutaronitrile (MDBGN) (286), hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) (102, 224, 263), isoeugenol (264, 279) and formaldehyde (287). The shaded values were considered as outliers.

Study	Number of patients	ED₁₀ ($\mu\text{g}/\text{cm}^2$)	95 % interval
MCI/MI	12	1.05	0.17–2.27
Formaldehyde	20	20.1	4.09–43.9
Nickel 1997	24	1.58	0.32–4.04
Nickel 1998	19	0.8	0.078–2.59

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Study	Number of patients	ED ₁₀ (µg/cm ²)	95 % interval
Nickel 1999	26	7.49	2.42–14.5
Nickel 2005	13	0.74	0.066–2.38
Nickel 2007	20	0.82	0.13–2.37
Cobalt 2005	11	0.44	0.033–1.3
Chromium	17	1.04	0.0033–5.55
Isoeugenol 2001	24	1.48	0.22–4.74
Isoeugenol 2005	13	0.23	0.0073–1.32
HICC 2003	18	0.85	0.062–3.26
HICC 2007	14	1.17	0.043–5.05
HICC 2009	17	0.66	0.052–2.35
MDBGN 2004	19	0.025	0.00021–0.19
MDBGN 2008	18	0.50	0.052–1.69

Note: The ED₁₀ value is the concentration which elicits an allergic reaction in 10% of a group of sensitised individuals under patch test conditions.

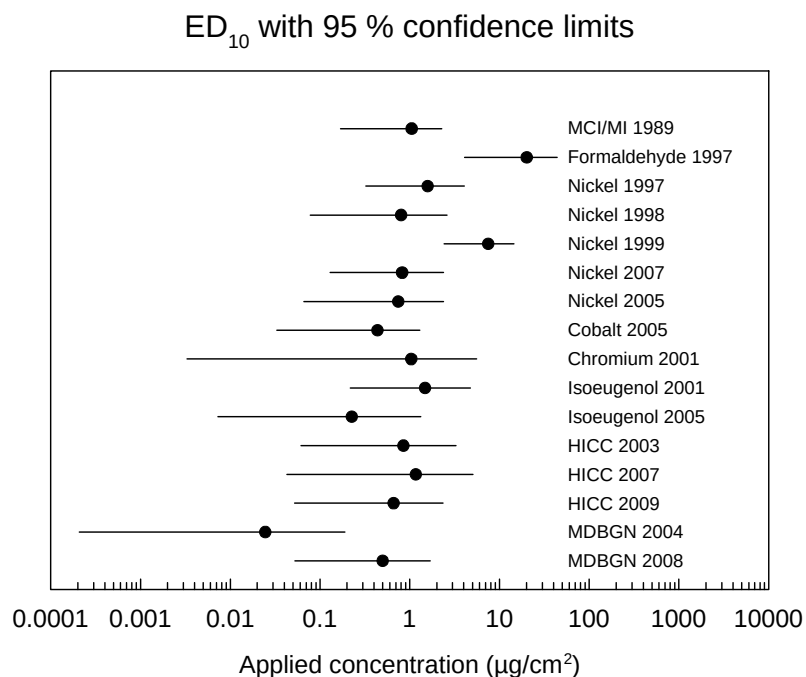


Figure 11-2: The threshold data with 95% confidence intervals from Table 11-6 presented graphically, after (280).

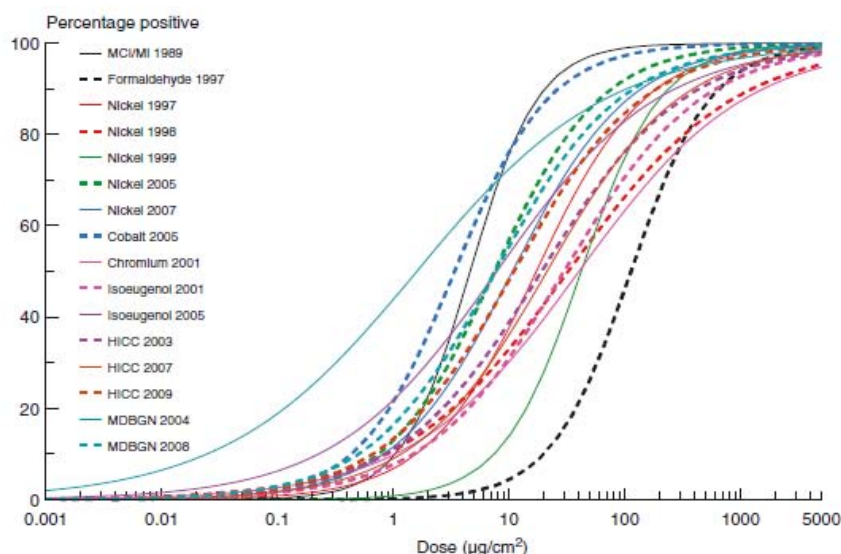


Figure 11-3: The fitted dose-response curves from the studies in Table 11-6, which are the basis for estimation of the ED10 value, after (280).

The meta-analysis above has shown that the median elicitation dose by patch testing for 10% of sensitised individuals was $0.8 \mu\text{g}/\text{cm}^2$. In the model data for the fragrance substances isoeugenol and HICC was included. The two studies on isoeugenol and the three studies on HICC gave an average ED10 value of $0.85 \mu\text{g}/\text{cm}^2$ and $0.89 \mu\text{g}/\text{cm}^2$ with a range 0.23-1.48. This means that even if the model was used for these substances individually the result would be very similar to the general threshold value.

The data from cinnamal and hydroxycitronellal studies was not incorporated in the model because: (i) serial dilution patch testing was done in petrolatum for cinnamal, making the dosing less exact; (ii) and only seven patients participated in the hydroxycitronellal study, while a criteria for inclusion in the model was ten participants (280).

According to the above calculations, a limit of $0.8 \mu\text{g}/\text{cm}^2$ for the product types of most importance for fragrance allergy corresponds to concentrations of 100 to 400 ppm (0.01-0.04%) for deodorants, perfume spray, hand and face lotions. For body lotion the general threshold was 0.16%. However, it does not seem meaningful in the context of contact allergy to distinguish between different types of creams, as a body cream would be applied with the hands and the relevant parameter in contact allergy is dose per area skin and not total dose.

A general threshold would have to take into consideration the uncertainties in quantification of exposure and safe thresholds as well as the possibilities of aggregate exposures and exposure to chemically similar substances. Therefore in setting one general threshold the product category carrying the highest risk of sensitisation and elicitation, which is deodorants, was chosen to drive the generation of the threshold. This means that a threshold of $0.8 \mu\text{g}/\text{cm}^2$ is equal to 0.01% or 100 ppm (see Table Table 11-1 and the related text), the lowest of the threshold values derived.

The approach taken by the SCCS is based on scientific evidence published in peer-reviewed journals (283)(284)(285)(286)(102, 224, 263)(264, 279)(287) in the past 20 years. The meta-analysis deriving the general threshold limit at $0.8 \mu\text{g}/\text{cm}^2$ limit has been published (280) in a peer-reviewed journal. The use of threshold limits based on elicitation data is a well established methodology which has been applied (with success) in EU to prevent further cases of induction and elicitation (primary and secondary prevention) in the case of nickel allergy, chromium in cement, chromium in shoes in

Germany, dimethyl fumurate in consumer items and also in part in IFRA guidelines e.g. concerning HICC.

The elicitation threshold model is based on 16 studies of 8 allergens, two of which are fragrance ingredients. It includes data from moderate to extreme allergens with a median EC3 value of 1.2.

The 11 fragrance allergens to which the limit is suggested to apply range from extreme to moderate with median EC3 value of 4.8, although in the case of coumarin an EC3 value could not be established.

Thus in general the potency profile of the fragrance substances of concern is not very different from those included in the model to provide the suggested general safe threshold.

The approach is targeting the relevant end-point, namely, allergic contact dermatitis. The mere consideration of potency of the allergen, according to the LLNA (EC3), is insufficient in identifying the size of the problems of contact allergy/allergic contact dermatitis. Additional information is needed from clinical and epidemiological studies, exposure assessment and dose-elicitation studies. For instance, the elicitation thresholds of e.g. HICC (EC3: 17.1) and isoeugenol (EC3: 0.54) are very similar (0.85 µg/cm² and 0.89 µg/cm², respectively) despite very different potencies. Both are frequent causes of contact allergy.

It should be noted that the general threshold is only suggested to be used for substances of concern if no specific data of sufficient quality exist to set an individual safe threshold. In cases where specific data of sufficient quality are available, these data should be used to set an individual safe threshold.

The general threshold is indicative of a safe level for the majority of sensitised individuals, but does not preclude that the most sensitive subset of the population may react upon exposure to the allergen. These levels are based on patch tests and take no account of anatomical sites of exposure, frequency of exposure or vehicle effects. Therefore, any limitations in exposures are not substitutes for providing information to the consumer about the presence of a substance in a product as a certain fraction of sensitised individuals will still need to avoid specific exposures.

Based on experience, limitations in exposure based on elicitation thresholds will, apart from helping the sensitised consumer, also significantly reduce the risk of induction. This is the case for nickel allergy, where the restrictions in the EU nickel directive are based on elicitation threshold, leading to a significant reduction in new cases of sensitisation in young women (288) and in a reduction in morbidity, i.e. elicitation (289). Another example is restriction of chromium VI in cement (290).

It is not possible to provide a safe threshold for natural extracts of concern, as no specific investigations exist, and the model providing the general use concentration limit (0.01%) has been based on chemicals only.

The SCCP concluded in 2004 that Chloroatranol and atranol, the main allergenic constituents of *Evernia prunastri* and *Evernia furfuracea*, should not be present in consumer products because they are extremely potent allergens (239). The persistently high frequency of contact allergy to *Evernia prunastri* and *Evernia furfuracea* noted in eczema patients does point to a persisting problem with exposure to the allergenic constituents.

11.3. Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) has been the most frequently reported individual fragrance chemical causing allergy since the 1999 opinion on fragrance allergy. In total, reports of about 1500 cases have been published in the scientific literature (see chapter 7.1 and Annex I to this opinion), while the second most

frequently reported individual chemical was cinnamal with around 350 published cases. Only a minority of the cases seen by clinicians is published and only a (small) proportion of those with allergic contact dermatitis seeks or has the possibility to seek medical attention.

Natural extracts such as *Myroxylon pereirae* and turpentine (oil) have been more frequently reported, but while HICC is a synthetic fragrance chemical, where the only source of exposure is fragrances, the natural extracts are used in many other contexts than fragrances/cosmetics.

Of patients tested by the Danish monitoring network of dermatologists 2.4% were found to be allergic to HICC in 2005-2008 (with no decreasing trend from 2003 to 2007 (291)) (for more studies see chapter 4.3.2); in 70% of the cases the reaction was of current relevance, i.e. causing disease (69). This is in agreement with the results of a recent German study with HICC, where 48 out of 51 patients (94.1%) with a positive patch test reaction to HICC also reacted in a repeated open application test, simulating normal use conditions of cosmetics containing HICC (105). In a Danish study 69% of 14 HICC allergic individuals developed allergic contact dermatitis from use of cosmetics containing HICC in realistic amounts (102).

On the basis of the high frequency of allergy to HICC, in 2003 the Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) recommended 0.02% (200 ppm) as maximum amount of HICC in cosmetic products (292). This has not been implemented and no restrictions apply in the Cosmetic Directive.

The fragrance industry, via the International Fragrance Association (IFRA), has its own safety guidelines. Up until 2003 HICC was used without any restriction; in 2003 a limit of 1.5% HICC in any kind of product was introduced. In 2008 this was changed according to the new risk assessment model (QRA) applied by the fragrance industry to different levels in 11 different product types derived from the QRA (see 11.1). Limits from 0.11% in lip products to 1.5% in hair styling products were set. In 2009 a further lowering was made of the limits by industry with the following reasoning: "The industry firmly believes and continues to support thresholds based on induction rather than elicitation. However, given the exceptional situation in Europe, the fragrance industry elected to take further restrictive action on this material" (293). An overview of the IFRA restrictions is given in the table below.

Table 11-7: Restriction for HICC independent of the QRA according to (293).

IFRA QRA Category	Product type that drives the category	Consumer exposure level 2003–2008 (%)	IFRA Standard July 2008 (%)	IFRA Standard July 2009 (%)
Category 1	Lip products	1.5	0.11	0.02
Category 2	Deodorants/ antiperspirants	1.5	0.15	0.02
Category 3	Hydroalcohols for shaved skin	1.5	0.60	0.2
Category 4	Hydroalcohols for unshaved skin	1.5	1.5	0.2
Category 5	Hand cream	1.5	1.0	0.2
Category 6	Mouthwash	1.5	1.5	Not applicable*
Category 7	Intimate wipes	1.5	0.3	0.02

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Category 8	Hair styling aids	1.5	1.5	0.2
Category 9	Rinse-off hair conditioners	1.5	1.5%	0.2%
Category 10	Hard surface cleaners	1.5	1.5%	0.2%
Category 11	Incidental or non-skin contact	15	Not restricted	Not restricted

Note: HICC Hydroxyisohexyl 3-cyclohexene carboxaldehyde.

QRA Quantitative risk assessment.

* Not applicable because HICC is not approved for flavour use.

As an update since the presentation of the pre-consultation version of the opinion, surveillance data on HICC from two European countries have become available, covering the period 2002-2011 (IVDK/Germany (294)) and 2003-2011 (Danish contact dermatitis group (295)), respectively. The first analysis identified a slight decrease, which was considered “not overwhelming in absolute terms”, namely, from 2.3% in 2002 to 2.1% in 2011 (crude prevalences, Figure 11-4). Thus, despite statistical significance, the decrease is too slight to be interpreted as relevant improvement. In the Danish study, some fluctuation around a mean prevalence of about 2.5% was noted, but no trend (Figure 11-5). It is reported that 74% of the positive reactions were regarded as clinically relevant.

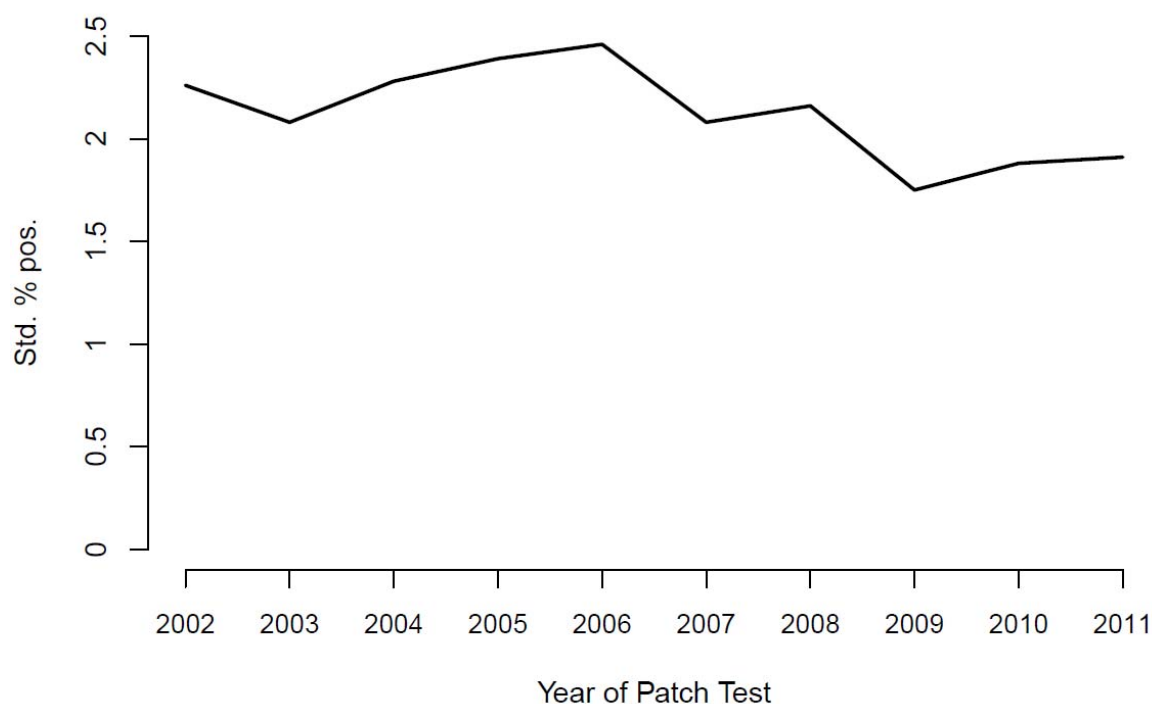


Figure 11-4: Time trend of hydroxyisohexyl 3-cyclohexene carboxaldehyde sensitisation prevalence [standardised prevalence of positives (%)] during 2002-2011. The decrease over time is statistically significant, after **(294)**.

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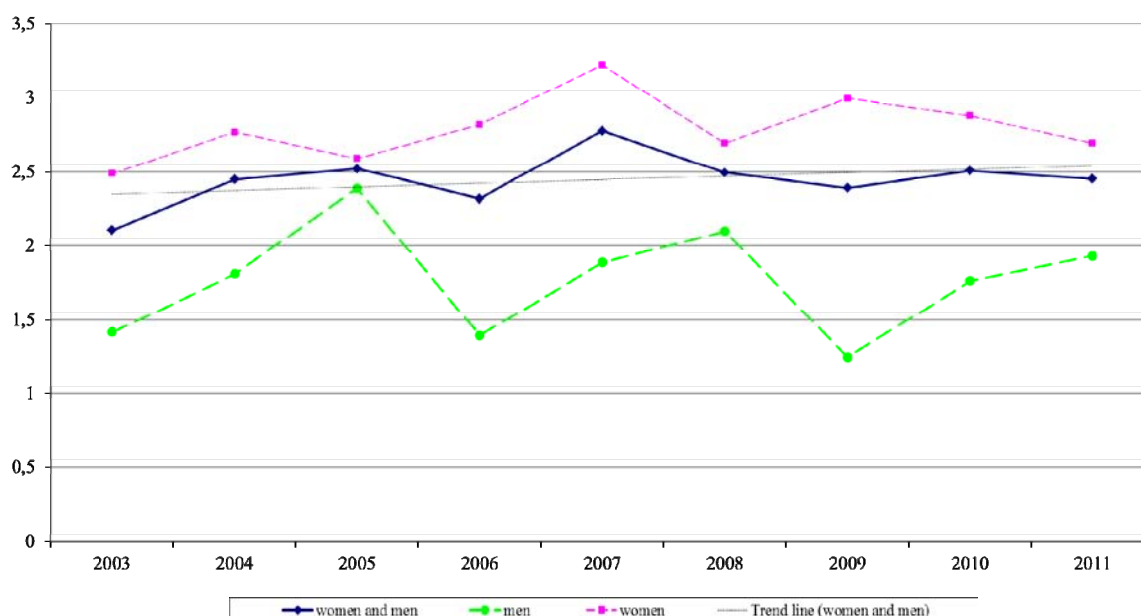


Figure 11-5: Prevalence of positive patch test reactions to hydroxyisohexyl 3-cyclohexene carboxaldehyde over time in 37 860 subjects tested by the Danish Contact Dermatitis Group (295).

11.4. Conclusion

- A dose-response relationship between exposure to contact allergens and induction of allergy (sensitisation) as well as elicitation is well established. This means that in principle, thresholds can be identified which are safe for the consumer.
- A model for dermal sensitisation quantitative risk assessment has been developed (QRA) and implemented by the fragrance industry. This model relies on thresholds, no effect or low-effect levels, established in healthy human volunteers and/or in animal experiments. The SCCP has previously reviewed this methodology and concluded that: "There is no confidence that the levels of skin sensitisers identified by the dermal sensitisation QRA are safe for the consumer."
- Elicitation data can provide thresholds indicative for the safe use of those substances which have already caused significant problems in the consumer. In this context, "safe use" means that the thresholds will protect the majority of consumers from allergic contact dermatitis, but does not preclude that the most sensitive subset of the population may react upon exposure to the allergen.
- Furthermore, based on experience from intervention studies, such thresholds will also be sufficiently low to protect (most of) the non-sensitised consumers from developing contact allergy.
- Elicitation levels have been studied specifically for the fragrance chemicals cinnamal, hydroxycitronellal and isoeugenol. These studies, however, are not adequate to derive safe thresholds for the individual substances directly from the data.
- In the absence of adequate substance specific data it is possible to use a general threshold. Based on a statistical analysis of the available data in the scientific literature, a threshold of $0.8 \mu\text{g}/\text{cm}^2$ was derived. This corresponds to 0.01% (100 ppm) limit in cosmetic products indicative for safe use.

- It is not possible to provide a safe threshold for natural extracts of concern, as no specific investigations exist and the model providing the general threshold (0.01%) has been based on individual chemicals only. However the maximum use concentration applies to the identified chemicals both if added as chemicals or as an identified constituent of a natural ingredient. This will also reduce the risk of sensitisation and elicitation from natural extracts.
- For substances for which there are no clinical data of concern, models such as the dermal sensitisation QRA approach may, after refinement and validation, be used to suggest a safe level of exposure prior to incorporation into products. However, aggregated exposures must be incorporated in the dermal sensitisation QRA model.
- HICC has for more than 10 years been recognized as an important allergen with more cases documented in the scientific literature than for any other fragrance chemical in this period. HICC has been shown to be a significant cause of disease as many of those with contact allergy to HICC had also reactions to cosmetics, which contained or were likely to contain HICC. Since 2003 attempts have been made by the fragrance industry to contain the outbreak of HICC allergy, but with no convincing success so far. Recent voluntary restrictions (recommendations to lower use concentrations, at least for some product types, to the level recommended by the SCCS in 2003) are not reflected in available evidence and are considered insufficient. The SCCS considers that the number of cases of HICC allergy documented over the last decade is exceptionally high and that continued exposure to HICC by the consumer is not considered safe, even at concentrations as low as 200 ppm. Therefore, HICC should not be used in consumer products in order to prevent further cases of contact allergy to HICC and to limit the consequences to those who already have become sensitized.
- The SCCP concluded in 2004 that chloroatranol and atranol, the main allergenic constituents of *Evernia prunastri* and *Evernia furfuracea*, should not be present in consumer products because they are extremely potent allergens. The persistently high frequency of contact allergy to *Evernia prunastri* and *Evernia furfuracea* noted in eczema patients does point to a persisting problem with exposure to the allergenic constituents, despite efforts to reduce the allergen content (296).

12. Data gaps and research needed

In the course of working on this opinion, the following points are highlighted as important data gaps, ordered by research area:

12.1. Clinical and epidemiological research

- Clinical data on more fragrance substances are needed to assess more fully the epidemiology of fragrance contact allergy and pin-point the culprit substances for induction and elicitation of contact allergy in man.
- Data from a broader range of EU countries on the clinical and epidemiological picture of fragrance contact allergy is needed, as difference in exposure and use habits are expected across Europe.
- A co-ordinated strategy for data collection should be developed.
- Very little is known about susceptible groups of the population, e.g. up 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.: +

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text
CARVONE	99-49-0 / 6485-40-1 / 2244-16-8	+ (r.t.)
CINNAMAL*	104-55-2	+++
CINNAMYL ALCOHOL*	104-54-1	+++
CITRAL*	5392-40-5	+++
CITRONELLOL*	106-22-9 / 1117-61-9 / 7540-51-4	++
COUMARIN*	91-64-5	+++
(DAMASCENONE) ROSE KETONE-4	23696-85-7	+ (r.t.)
alpha-DAMASCONE (TMCHB)	43052-87-5 / 23726-94-5	++
cis-beta-DAMASCONE	23726-92-3	+
delta-DAMASCONE	57378-68-4	+
DIMETHYLBENZYL CARBINYL ACETATE (DMBCA)	151-05-3	+
EUGENOL*	97-53-0	+++
FARNESOL*	4602-84-0	++ - +++
GERANIOL*	106-24-1	+++
HEXADECANOLACTONE	109-29-5	+ (r.t.)
HEXAMETHYLINDANOPYRAN	1222-05-5	++
HEXYL CINNAMAL*	101-86-0	++
HYDROXYISOHEXYL CARBOXALDEHYDE (HICC)* 3-CYCLOHEXENE	31906-04-4 / 51414-25-6	++++
HYDROXYCITRONELLAL*	107-75-5	+++
ISOEUGENOL*	97-54-1	+++
alpha-ISOMETHYL IONONE*	127-51-5	++
(DL)-LIMONENE*	138-86-3	++ (non-ox.); +++ (ox.)
LINALOOL*	78-70-6	++ (non-ox.) +++ (ox.)
LINALYL ACETATE	115-95-7	+ (non-ox.) ++ (ox.)
MENTHOL	1490-04-6 / 89-78-1 / 2216-51-5	++
6-METHYL COUMARIN	92-48-8	++
METHYL 2-OCTYNOATE*	111-12-6	++
METHYL SALICYLATE	119-36-8	+
3-METHYL-5-(2,2,3-TRIMETHYL-3-	67801-20-1	++ (r.t.)

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

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

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

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

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

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

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

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

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

n.a.: not applicable (natural mixture)

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

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

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

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

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

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

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

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

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

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

*including their respective esters

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

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

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

Conclusions - Question 1

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

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

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

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

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

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

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

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

13.2. Question 2

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

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

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

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

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

If no adequate data are available, for substances posing a high risk to the consumer (like the 12 listed in Table 13-5), the use of a general threshold may be considered. A threshold of 0.8 µg/cm² has been derived based on a statistical analysis of the available data in the scientific literature, including two fragrance allergens. This corresponds to 0.01% (100 ppm) limit in cosmetic products indicative for safe use. This approximation may hold for weak to strong allergens. However, some strong and extreme sensitisers may require lower individual thresholds. As an example, chloroatranol, present in the natural product *Evernia prunastri* and in *Evernia furfuracea*, has been shown to have an elicitation threshold of 0.0004 µg/cm² under experimental conditions similar to those yielding above results. On the other hand, for very weak sensitisers, this generic threshold may be too conservative.

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

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

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

Conclusions - Question 2

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

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

The dose elicitation studies available indicate that a general level of exposure of up to 0.8 µg/cm² (0.01%) may be tolerated by most consumers with contact allergy to fragrance allergens. The SCCS considers that this level of exposure could be efficient in limiting elicitation unless there is substance specific data, either experimental or clinical, to the contrary.

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

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

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

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

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

13.3. Question 3

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

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

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

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

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

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

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

Based on the clinical data, oxidised limonene and oxidised linalool are allergens of high concern (Table 13-5) which pose a high risk of sensitisation to the consumer. For these substances the presence of the oxidised fraction represented by the peroxide content should not be higher than 10 ppm. Alternatively, the suggested general threshold dose/area of 0.8 µg/cm² (100 ppm in cosmetic products) could be applicable to the total oxidised fraction, i.e. not only peroxides but also secondary oxidation products such as aldehydes and epoxides.

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

Table 13-6: Known prehaptens and prohaptens.

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

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

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

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

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

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

Conclusions - Question 3

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

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

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

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

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

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

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

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

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

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

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

14. List of abbreviations

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

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

15. References

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Annex I - Catalogue of fragrance allergens**Contents**

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Single chemicals

Often, results with the single constituents of the FM I or, yet more rarely, FM II, are presented in one paper. As the main ordering of this annex is by allergen, core information on these studies is presented in a tabular format and referenced by a unique acronym in the single sections, to avoid redundancy. Regarding nomenclature, terms which are often not officially an INCI Name but Perfuming Name as listed by CosIng are used. "Current Regulation" refers to the EU Cosmetics Directive only.

Table 55: Background information on studies reporting results with (all) single constituents of the FM I (**amyl cinnamal, cinnamyl alcohol, cinnamal, eugenol, geraniol, hydroxycitronellal, isoeugenol, EVERNIA PRUNASTRI**)

Reference	Country	Study period, Patients	Comments by reviewers
Larsen 2002 c (1)	7 industrial countries worldwide	Prior to 2002 n=218 patients with known contact allergy to fragrance ingredients	Test concentrations identified as non-irritating in serial dilution testing in 20 healthy volunteers
Utrecht 1999 (2)	Utrecht, The Netherlands	1994-1998 n=757 patients with suspected ACD to cosmetics	All patients tested with FM I and single constituents
Sheffield 1999 (3)	UK	1994-1995 n=744, 40 of these positive to FM I and tested with single constituents	
IVDK 2007 (4)	Germany + one centre in Austria and Switzerland each	01/2003 – 12/2004, n=1658 to 21325, see text, consecutive patients	
Hungary 2002 (5)	Hungary, multicentre study,	1998-1999, n=3604 patients	recruitment not clear, presumably consecutive patients
Groningen 2009 (6)	Groningen, The Netherlands	04/2005-06/2007 n=320	patients selected according to history or site suspicious of contact allergy to fragrance ingredients
IVDK 2010 (7)	Germany, Switzerland and one centre in	2005-2008 n=36961 tested with FM I, n=4167 with FM II and	

	Austria	all SC	

Table 56: Results of PTing with single constituents of the FM I in patients positive to the FM I (as percent)

N(pos) to FM I, ref.	<i>Evernia prun.</i>	Isoeu g.	Hydroxy citron.	Cinna mal	Cinnamy I alcohol	Eugen ol	Gera-niol	Alpha-amyl cinnam al
N=160 (5)	13.1%	14.8 %	2.5%	8.1%	20.6%	8.8%	7.5%	5.0%
N= 991 (8)	18.4%	11.2 %	10.1%	6.1%	6.1%	6.6%	4.6%	2.4%
N=50 (2)	19.6%	14.3 %	8.9%	8.9%	7.1%	5.4%	2.7%	0%
n=40 Sheffield 1999 (3)	30%	20%	2.5%	12.5 %	10%	5%	0%	0%
N=226 Coimbra 2000 (9)	22.1%	19.9 %	6.6%	13.3 %	7.9%	14.6 %	8.4%	4.4%
N=655 IVDK 2010 (7)	29.8%	18.0 %	12.8%	11.6 %	9.6%	6.7%	4.7%	2.8%

Table 57: Background information on studies reporting results with (all) single constituents of the FM II (**citronellol, citral, coumarin, hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), Farnesol, alpha-Hexyl-cinnamic aldehyde**)

Reference	Country	Study period, Patients	Comments by reviewers
IVDK 2007 (4)	Germany + one centre in Austria and Switzerland each	01/2003 – 12/2004, n=1658 to 21325, see text, consecutive patients	
EU 2005 (10)	6 European centres	10/2002 – 06/2003, n=1701	Applied in consecutive patients
Groningen 2009 (6)	Groningen, The Netherlands	04/2005-06/2007 n=320	patients selected according to history or site suspicious of contact allergy to fragrance ingredients

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

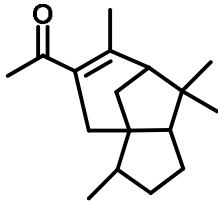
Table 58: Background information on studies reporting results with several fragrance compounds not, or only partly, corresponding to mixes (later created) or with essential oils

Reference	Country	Study period, Patients
deGroot 2000 (12)	The Netherlands (multicentre)	09/1998-04/1999 n=1825 consecutive patients
An 2005 (13)	South Korea (multicentre)	04/2002 – 06/2003 n=422 consecutive patients
Sugiura 2000 (14)	Nagoya, Japan	1990-1998 n=1483 patients with suspected cosmetic dermatitis
Frosch 1995 (15)	11 European depts.	Prior to 1995 n=1069 consecutive patients
Frosch 2002 a (16)	6 European depts.	10/1997-10/1998 n=1855 consecutive patients
Frosch 2002 b (17)	6 European depts.	Prior to 2002 n=1606 consecutive patients
Coimbra 2000 (9)	Portugal	07/1989-06/1999 n=226 with FM I SC n=67 also with other fragrances
Larsen 1977 (18)	US	1977 n=20 “perfume-sensitive patients”
Larsen 2001 (19)	worldwide multicentre	? (prior to 2001) n=178 patients with known contact allergy to fragrance ingredients
Belsito 2006 (20)	North American (5 US, 1 Canadian) depts.	2003 n=1603 patients
NACDG 2009 (21)	US and Canada	2005-2006 n= 4454 patients
Wöhrl 2001 (22)	“FAZ” clinic Vienna	1997-2000 n=747 of 2660 consecutive patients tested with special series
EECDRG 1995 (15)	European, multicentre	Different fragrances, tested in 2 concentrations, in sets of about 100 patients each in different centres
Goossens 1997 (23)	Leuven, Belgium	1978-1987 n=111 “Japanese perfume series” (highly selected patients)

Opinion on fragrance allergens in cosmetic products

Reference	Country	Study period, Patients
Malten 1984 (24)	Dutch multicentre	N=182 patients with suspected cosmetic dermatitis tested with 22 fragrance compounds
DeGroot 1985 (25)	Dutch	N=179 patients with suspected cosmetic dermatitis tested with 16 fragrance compounds
Rudzki 1976 (26)	Warsaw, Poland	N=200 consecutive patients
Rudzki 1986 (27)	Warsaw, Poland	N=86 patients of 299 (of 5315) patients with positive reaction to FM I tested with essential oils series
Santucci 1987 (28)	Rome, Italy	N=1500 consecutive patients; n=63 reacting positively to FM I re-tested with extended fragrance series
Nakayama 1974 (after (29))	Japan	N=183 patients with cosmetic dermatitis
IVDK 2010c (30)	Germany, Switzerland and one centre in Austria	15682 patients tested with at least one essential oil in different test series
Trattner/David (31)	Tel Aviv, Israel	N=641 consecutive patients

Catalogue of single chemicals evaluated

ACETYLCEDRENE	
CAS # 32388-55-9	
EC # 251-020-3	
1-[(3R,3aR,7R,8aS)-2,3,4,7,8,8a-Hexahydro-3,6,8,8-tetramethyl-1H-3a,7-methanoazulen-5-yl]-ethanone	
Other names 1-(2,3,4,7,8,8a-Hexahydro-3,6,8,8-tetramethyl-1H-3a,7-methanoazulen-5-yl)-, [3R-(3a,3aβ,7β,8aα)]-Ethanone; 1H-3a,7-Methanoazulene, Ethanone deriv.; Acetyl-α-cedrene; Lixetone; Vertofix	

Current regulation: /

Clinical

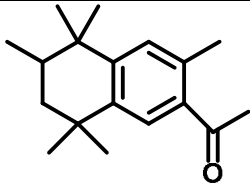
data:

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 none to 5% "Vertofix ®" in pet., tested in 100 consecutive patients in Stockholm, were observed (15). In a case report, a 28-year-old patient with axillary dermatitis after using 2 different deodorants tested positive not only to HICC, but also to acetyl cedrene (tested 10.8% in diisopropylene glycol (20 healthy controls negative) (32). In this case report it is stated that "Acetyl cedrene (Vertofix Coeur) is a complex reaction mixture of which a principal constituent is methyl cedryl ketone".

Additional

information:

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

6-ACETYL-1,1,2,4,4,7-HEXAMETHYLTETRALINE	
CAS # 21145-77-7	
EC # 216-133-4 / 244-240-6	
1-(5,6,7,8-Tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthalenyl)-ethanone	
AHMT (perfume), AHTN, Extralide, Fixolide, Musk tonalid, NSC 19550, Tentarome, Tetralide, Tonalid, Tonalide.	

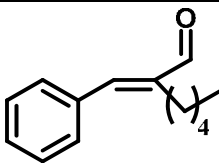
Current regulation: Annex III, part 1, entry 182

Clinical

data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% "Tonalide ®" in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15).

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

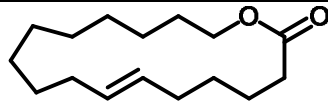
AMYL CINNAMAL	
CAS # 122-40-7	
EC # 204-541-5	
2-(Phenylmethylene)-heptanal	
Cinnamaldehyde, α-amyl- (4CI); Cinnamaldehyde, α-pentyl- (6CI,7CI,8CI); 2-(Phenylmethylene)heptanal; 2-Benzylideneheptanal; Amylcinnamaldehyde; Amylcinnamic acid aldehyde; Amylcinnamic aldehyde; Flomine; Jasminal; Jasminaldehyde; Jasmine aldehyde; NSC 6649; Pentylcinnamaldehyde; α-Amyl-β-phenylacrolein; α-Amylcinnamal; α-Amylcinnamaldehyde; α-Pentylcinnamaldehyde	

Current regulation: Annex III, part 1, entry 67

Clinical data:
In the “background information” section of the 1999 opinion (33), amyl cinnamal (synonymous: alpha amyl cinnamaldehyde) has been classified as frequently reported contact allergen because it has been identified as a cause of allergic reactions in persons with eczema from cosmetic products.

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded n=4, i.e., 0.2% (95% CI: 0.1 – 0.5%) positive reactions to this compound (1% pet.) in 2062 consecutively PTed patients (4). In the Groningen 2009 study, no positive reactions to this allergen, tested at 2% pet., were observed (6). The Larsen 2001 study yielded 2.3% positive reactions in 178 patients with known contact allergy to fragrance ingredients (test concentration: 5% pet.) (19). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=2 (0.3%) positive reactions to amyl cinnamal (22). The IVDK 2010 study, 0.26% (95% CI: 0 – 0.60%) of 1214 consecutively tested patients reacted to the compound, while 0.61% (95% CI: 0.36 – 0.86%) of 4375 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7).

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

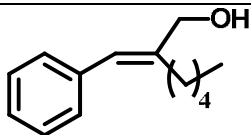
AMBRETTOLIDE	
CAS # 7779-50-2	
EC # 231-929-1	
Oxacycloheptadec-7-en-2-one	
1-Oxa-7-cycloheptadecen-2-one; 16-Hydroxy-6-hexadecenoic acid lactone; 16-Hydroxy-6-hexadecenoic acid ω-lactone	

Current regulation: /

Clinical data:
The Larsen 2001 study, using omega-6-hexadecenlactone (HDL, 5% pet.) as test concentration, diagnosed 3.4% positive reactions in 178 patients with known contact allergy to fragrance ingredients (19).

Additional information:

Ambrettolide is 1 of 2 components of Ambrette seed oil (obtained from *Hibiscus abelmoschus* L., *Malvaceae*) responsible for the musk odour. In Surburg/Panten, the compound has the chemical name (Z)-7-hexadecen-16-olide (or Hexadec-7-en-16-olide according to CosIng), CAS 123-69-3 (34).

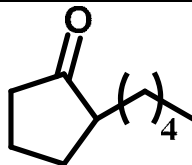
AMYL CINNAMYL ALCOHOL	
CAS # 101-85-9	
EC # 202-982-8	
2-(Phenylmethylene)-heptan-1-ol,	
2-Benzylidene- (6Cl,8Cl)1-heptanol; 2-Amyl-3-phenyl-2-propen-1-ol; 2-Benzylidene-1-heptanol; 2-Pentyl-3-phenyl-2-propen-1-ol; Buxinol; α-Amylcinnamic alcohol; α-Amylcinnamyl alcohol	

Current regulation: Annex II, Part 1, entry 74

Clinical data:
In the “background information” section of the 1999 opinion, amyl cinnamyl alcohol is mentioned to cross-react with amyl cinnamal. Moreover, this compound has been identified as a cause of allergic reactions in a notable number of persons with eczema from the use of cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.4% (95% CI: 0.1 – 0.7%) positive reactions in 1977 consecutively PTed patients (4). The IVDK 2010 study, 0.79% (95% CI: 0.54 – 1.04%; percentages standardised for age and sex) of 5650 patients PTed reacted to the compound (7). In the Groningen 2009 study, 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen (6).

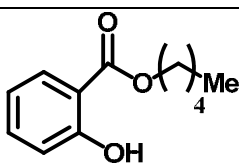
Additional information: A RIFM review is available (35) where selected clinical studies published until 1994 were considered.

AMYLCYCLOPENTANONE	
CAS # 4819-67-4	
EC # 225-392-2	
2-Pentylcyclopentanone	
2-Pentyl-1-cyclopentanone; 2-Pentylcyclopentanone; 2-Pentylcyclopenten-1-one; 2-n-Amylcyclopentanone; 2-n-Pentyl cyclopentanone; Delphone	

Current regulation: /

Clinical data:
In the Larsen 2001 study, none of 178 patients with contact allergy to fragrance ingredients reacted positively to this ingredient, PTed at 5% pet. (19).

Additional information: /

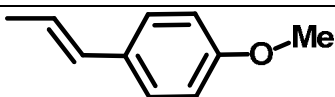
AMYL SALICYLATE	
CAS # 2050-08-0	
EC # 218-080-2	
Pentyl-2-hydroxybenzoate	
Amyl ester salicylic acid, (4CI); Pentyl ester salicylic acid, (6CI,8CI); 2-Hydroxybenzoic acid pentyl ester; Amyl salicylate; NSC 403668; NSC 44877; NSC 46125; Pentyl salicylate	

Current regulation: /

Clinical data:

In the Frosch 2002 a study, a total of n=3 (0.2%) had positive PT reactions (16). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% amyl salicylate and 1 positive reaction to 5% amyl salicylate were observed in 100 consecutive patients patch tested in Stockholm (15).

Additional information:
A RIFM review is available (36). It is a “top 100” substance (IFRA, pers. comm.2010)

trans-ANETHOLE	
CAS # 4180-23-8	
EC # 224-052-0 / 203-205-5	
1-Methoxy-4-(1E)-1-propen-1-yl-benzene	
(E)-p-Propenyl-anisole (8CI); (E)-1-Methoxy-4-(1-propenyl)-benzene; 1-Methoxy-4-(1E)-1-propenyl-benzene (9CI); (E)-1-(4-Methoxyphenyl)propene; (E)-1-p-Methoxyphenylpropene; (E)-Anethol; (E)-Anethole (REACH, EINECS); E-Anethole (INCI); 1-Methoxy-4-[(1E)-1-propenyl]benzene; (E)-1-Methoxy-4-(1-propenyl)-benzene (CosIng); NSC 209529; trans-1-(4-Methoxyphenyl)-1-propene; trans-1-(p-Methoxyphenyl)-1-propene; trans-1-(p-Methoxyphenyl)propene; trans-1-p-Anisylpropene; trans-4-(1-Propenyl)anisole; trans-Anethol; trans-Anethole; trans-p-Anethole; trans-p-Methoxy-β-methylstyrene	

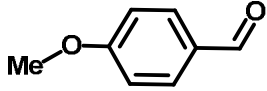
Current regulation: /

Clinical data:

A case of a 64 year old patient, who developed severe cheilitis and a loss of taste has been described (37). Both were reversible after the cessation of use of previous toothpastes. The patch test was strongly positive to anethole (isoform not given) 5% pet.; this was found an ingredient of the causative toothpaste. Two cases of occupational allergic contact dermatitis occurring in a traditional cake factory due to anise oil have been described, both testing (strongly) positive to anise oil (5% o.o.) and anethole (5% pet.) (38).

Additional information:
It is a “top 100” substance (IFRA, pers. comm.2010). trans-Anethole can be purified from star anise oil (34, 39), see 3.2., and is the main component of anise, star anise

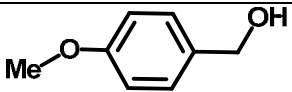
and fennel oils (38)

ANISALDEHYDE	
CAS # 123-11-5	
EC # 204-602-6	
4-Methoxy-benzaldehyde	
p-Methoxybenzaldehyde; p-Anisaldehyde; 4-Anisaldehyde; Aubepine; Crategine; NSC 5590; Obepin; p-Anisic aldehyde; Anisic aldehyde; p-Formylanisole.	

Current regulation: /

Clinical data: /

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

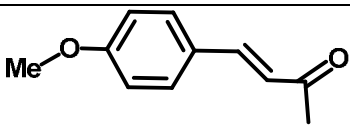
ANISYL ALCOHOL	
CAS # 105-13-5	
EC # 203-273-6	
4-Methoxy-benzenemethanol	
p-Methoxy-benzyl alcohol (8CI); (4-Methoxyphenyl)methyl alcohol; 4-(Hydroxymethyl)anisole; 4-(Methoxyphenyl)methanol; 4-Methoxy- α -hydroxytoluene; 4-Methoxybenzenemethanol; 4-Methoxybenzyl alcohol; Anise alcohol; Anisic alcohol; NSC 2151; [4-(Methoxy)phenyl]methanol; p-(Methoxyphenyl)methanol; p-Anisalcohol; p-Anisyl alcohol; p-Methoxybenzyl alcohol	

Current regulation: Annex III, part 1, n° 80

Clinical data:
In the “background information” section of the 1999 opinion, anisyl alcohol is classified as “less frequently reported allergen”; 2 studies were identified where 3 and 4 cases, respectively, with cosmetic dermatitis due to contact allergy to anisyl alcohol had been reported (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded $n=1$, i.e., 0.1% (95% CI: 0.00 – 0.3%) positive reactions in 2004 consecutively PTed patients, patch test concentration: 1% pet. (4). Similar results were obtained in the following period, with $n=1$ (and $n=3$ irritant and $n=6$ doubtful) reactions in 986 patients tested with 1% in pet. (30). In the Groningen 2009 study, no positive reactions to this allergen, tested at 5% pet., were observed in 320 patients (6). This test concentration has been regarded as relatively high by Hostynek and Maibach (40). The test concentration of Anisyl Alcohol has been further validated by Bruze et al. and 10% in pet was recommended as a non-irritant concentration for routine investigations (40a).

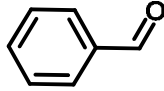
Additional information: /

ANISYLIDENE ACETONE	
CAS # 943-88-4	
EC # 213-404-9	
4-(4-Methoxyphenyl)-3-Buten-2-one	
1-(p-Methoxyphenyl)-1-buten-3-one; 4-(4-Methoxyphenyl)-3-buten-2-one; 4-(p-Methoxyphenyl)-3-buten-2-one; 4-Methoxybenzalacetone; 4-Methoxybenzylideneacetone; 4-Methoxystyryl methyl ketone; 4'-Methoxybenzylideneacetone; Anisalacetone; Methyl p-methoxystyryl ketone; NSC 31752; NSC 7946; p-Anisalacetone; p-Methoxybenzalacetone; p-Methoxybenzylideneacetone; p-Methoxystyryl methyl ketone	

Current regulation: Annex III, part 1, n° 443

Clinical data:
In the Malten 1984 study, 1.1% of 182 patients displayed a positive PT reaction to anisylidene acetone 2% pet. (24)

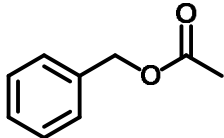
Additional information: /

BENZALDEHYDE	
CAS # 100-52-7	
EC # 202-860-4	
Benzaldehyde	
Artificial Almond Oil; Benzaldehyde FFC; Benzenecarbonal; Benzenecarboxaldehyde; Benzoic acid aldehyde; Benzoic aldehyde; NSC 7917; Phenylformaldehyde; Phenylmethanal	

Current regulation: /

Clinical data:
 In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=3 (0.4%) positive reactions to benzaldehyde 5% pet. (22). The IVDK 2010 study, 6 weak positive reactions were observed, i.e., 0.16% (95% CI: 0.03 – 0.29%; percentages standardised for age and sex) of 2820 patients PTed reacted to the compound (7). A review is available in the Int. J. Toxicol. (41). In the case of a 19 year old pastry maker, Seite-Bellezza et al. report on immediate reactions to MP, cinnamal and benzaldehyde (tested at 5% pet.) subsiding after a few hours, in line with the patient's history (42).

Additional information: /

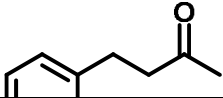
BENZYL ACETATE	
CAS #140-11-4	
EC # 205-399-7 / 202-940-9	
Benzyl acetate	
Benzyl ester acetic acid; Benzyl alcohol, acetate (6CI); (Acetoxymethyl)benzene; Benzyl ethanoate; NSC 4550; Phenylmethyl acetate; Methyl Phenylacetate; α-Acetoxytoluene ; Methyl alpha-Toluate	

Current regulation: /

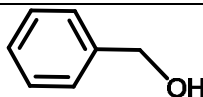
Clinical data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% benzyl acetate in pet., tested in 100 consecutive patients in Odense, DK, were observed (15). Benzyl acetate is a component of several natural mixtures, for example a major constituent of Narcissus abs., and a minor constituent of Jasmine abs. (17).

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

BENZYL ACETONE	
CAS # 2550-26-7	

EC # 219-847-4	
4-Phenyl-2-butanone	
4-Phenylbutan-2-one (REACH, EINECS); Benzylacetone; Methyl 2-phenylethyl ketone; Methyl phenethyl ketone; NSC 44829; NSC 813M; Phenethyl methyl ketone; 1-Phenyl-3-butanone; 2-Phenylethyl methyl ketone	
Current regulation: /	
Clinical data: /	
Additional information: It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (43).	

BENZYL ALCOHOL	
CAS # 100-51-6	
EC # 202-859-9	
Phenylmethanol	
Benzyl alcohol; (Hydroxymethyl)benzene; Benzenecarbinol; Benzylic alcohol; NSC 8044; Phenylcarbinol; Benzenemethanol; Phenylmethyl alcohol; Sunmorl BK 20; TB 13G; α-Hydroxytoluene; α-Toluenol	
Current regulation: Annex III, part 1, n° 45; Annex VI, part1, n ° 34	

Clinical data:
In the “background information” section of the 1999 opinion, benzyl alcohol is classified as allergen frequently causing allergic reactions. It has been found to cause allergic reactions in 1.2 to 15% of patients with eczema from cosmetic products (33). A CIR expert panel review is available in the Int. J. Toxicol. (44).

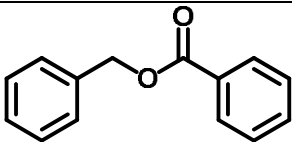
Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.3% (95% CI: 0.1 – 0.7%) positive reactions in 2166 consecutively PTed patients (4). In the Groningen 2009 study, n=1, i.e. 0.3% (95% CI: 0.01 – 1.7%) had positive reactions to this allergen (6).

Both in terms of case reports (45-47) and clinical epidemiology data (0.22 % [95% CI: 0.16 – 0.28%] positive tested with benzyl alcohol in the context of a “topical drugs” series, n=26448 (7)) the relevance of this alternative exposure is highlighted. In a study from Alicante, Spain, 86 selected patients were tested with benzyl alcohol, yielding 2 positive reactions (48).

After application of saline soaks preserved with benzyl alcohol onto his stasis dermatitis, a 53 year old patient developed a rash, which was, according to test results obtained by J. D. Guin and J. Goodman, at least partly due to an immediate hypersensitivity to benzyl alcohol, as verified by an intense urticarial reaction at the test site lasting several days (49). According to 2 cases reported by A. A. Fisher, PT-proven, relevant delayed type hypersensitivity is not associated with immediate reactions in scratch or intradermal tests (50). D. W. Shaw describes a patient with allergic contact dermatitis caused by benzyl alcohol in a hearing aid impression material and in topical medications (51). Another contribution points to covert exposures to benzyl alcohol even in products labelled “fragrance free” (52) probably because benzyl alcohol is used as preservative, or an essential oil containing benzyl alcohol is used as cosmetic ingredient.

Additional information:

Benzyl alcohol is a component of several natural mixtures, including Myroxylon pereirae resin, which have been used for extraction, but is nowadays synthesised (53). It is permitted in certain foodstuffs (liquors: < 100 mg/l, sweets and cakes: < 250 mg/kg) under the coding “E 1519” (http://www.zusatzstoffe-online.de/zusatzstoffe/317.e1519_benzylalkohol.html, last accessed 2009-11-27). In addition to being a fragrance compound (which may be used, even in relatively high concentration, to scent topical medications (54)), benzyl alcohol is used as antioxidant in topical therapeutics or cosmetics. The German “Rote Liste” (<http://www.rote-liste.de>, last accessed 2009-11-11), for instance, lists 205 specialties containing benzyl alcohol. Benzyl alcohol may be used up to 1.0% as a preservative in cosmetic products according to the Cosmetic Directive 76/768/EEC

BENZYL BENZOATE	
CAS # 120-51-4	
EC # 204-402-9	
Benzyl benzoate	
Benzyl ester benzoic acid; Ascabin; Ascabiol; Benylate; Benzyl benzenecarboxylate; Benzyl benzoate; Benzyl phenylformate; Benzylets; Colebenz; NSC 8081; Nicca Sunsolt LM 7EX; Novoscabin; Pelemol B66; Peruscabin; Phenylmethyl benzoate; Scabagen; Scabanca; Scabcare BB; Scabide; Scabiozon; Scobenol; Vanzoate; Venzonate	

Current regulation: Annex III, part 1, n° 85

Clinical data:

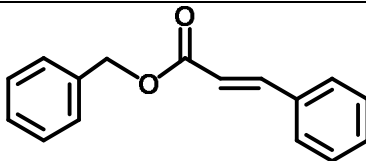
In the “background information” section of the 1999 opinion, benzyl benzoate is classified as “less frequently reported allergen”; in several studies, only single cases had been reported in each, except for patients sensitive to MP (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded n=1, i.e., 0.1% (95% CI: 0.00 – 0.3%) positive reactions in 2003 consecutively PTed patients, test concentration 1% pet. (4). In the subsequent period (2005-2008), n=1062 patients were tested in the IVDK 2010 study, with no positive reactions (7). In the Groningen 2009 study, no positive reactions to this allergen, tested at 5% pet., were observed in 320 patients (6). Thus, the pooled proportion of positive patch test reactions is 1 / 3385 (0.03%, exact upper 1-sided 95% CI: 0.14%)

Additional information:

Benzyl benzoate naturally occurs in MP resin and ylang-ylang oil. Nowadays it is synthesised and used for a variety of purposes (53). These include use as a scabicide (one brand specialty on the German market, using a concentration of 10% for children and 25% for adults), possibly with some differences among European countries. In France, a combination of benzyl benzoate 10% and sulfiram 2% is reported to be used most often (55). Hausen et al. review the older literature and mention a study identifying 1 sensitised patient in 73 patients treated for scabies (details not given) (53). According to the mandatory factsheet (see PDF “benzylbenzoate_infosheet_DE.pdf”) dermatitis after anti-scabies treatment is “rare”, in a range between 1:1000 and 1:10000.

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

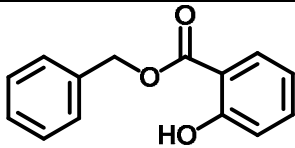
BENZYL CINNAMATE	
CAS # 103-41-3	
EC # 203-109-3	
Benzyl 3-phenylprop-2-enoate	
Benzyl ester cinnamic acid; 3-phenyl-phenylmethyl ester 2-propenoic acid; 3-Phenyl-2-propenoic acid benzyl ester; Benzyl 3-phenylpropenoate; Benzyl γ-phenylacrylate; Cinnamein; NSC 11780; NSC 44403	

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).

<i>BENZYL SALICYLATE</i>	
CAS # 118-58-1	
EC # 204-262-9	
Benzyl 2-hydroxybenzoate	
Salicylic acid, Benzyl ester; Benzoic acid, 2-Hydroxy-, phenylmethyl ester; Benzyl o-hydroxybenzoate; NSC 6647	

Current regulation: Annex III, part 1, n° 75

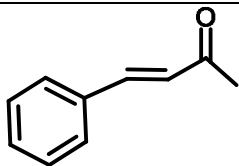
Clinical data:

In the “background information” section of the 1999 opinion (33), benzyl salicylate is classified among the frequent allergens, with 0.2 to 10% of patients with eczema from cosmetic products testing positively. In one study, benzyl salicylate accounted for 75% of reactions to commercial products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded n=2, i.e. 0.1% (95% CI: 0.01 – 0.4%) positive reactions in 2041 consecutively PTed patients (test concentration 1% pet.) (4). The IVDK 2010 study, 2 of 3775 patients PTed reacted weakly positive to the compound (7). In the Groningen 2009 study, n=1, i.e. 0.3% (95% CI: 0.01 – 1.7%) had positive reactions to this allergen, tested at 2% pet. (6). In the deGroot 2000 study, 10 of 1825 consecutive patients tested positive to benzyl salicylate (2% pet.), of these, 3 were not detected by the FM I (12). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=3 (0.4%) positive reactions (22). Trattner/David found 2 positive cases in 641 consecutive eczema patients (31). In a study from Alicante, Spain, 86 selected patients were tested with benzyl salicylate, yielding 2 positive reactions (48).

Additional information:

It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010). A RIFM review is available, including internal results on, e.g. HRIPT, and a review of LLNA results, where benzyl salicylate is classified as “weak” allergen (57).

BENZYLIDENEACETONE	
CAS # 122-57-6	
EC # 204-555-1	
4-Phenyl-3-buten-2-one	
4-Phenylbut-3-en-2-one; 2-Butenone, 4-Phenyl- (2CI); Ketone, Methyl styryl (7CI); 1-Phenyl-1-buten-3-one; 2-Phenylethenyl methyl ketone; 2-Phenylvinyl methyl ketone; 4-Phenyl-3-buten-2-one; 4-Phenyl-3-butene-2-one; 4-Phenylbutenone; Acetocinnamone; Benzalacetone; Benzylideneacetone; Methyl 2-phenylvinyl ketone; Methyl phenylvinyl ketone; Methyl styryl ketone; Methyl β-styryl ketone; NSC 5605; Styryl methyl ketone	

Current regulation: Annex II, n° 356

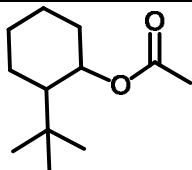
Clinical

data:

In the Malten 1984 study, none of 182 patients displayed a positive PT reaction to

benzylidene acetone 0.5% pet. (24).

Additional information: /

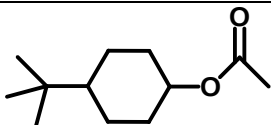
2-TERT-BUTYLCYCLOHEXYL ACETATE	
CAS # 88-41-5	
EC # 201-828-7	
2-(1,1-dimethylethyl)cyclohexyl acetate	
Cyclohexanol, 2-(1,1-dimethylethyl)-, acetate ; Cyclohexanol, 2-Tert-butyl-, acetate; 2-Tert-Butylcyclohexanol acetate; Verdox; o-Tert-Butylcyclohexyl acetate	

Current regulation: /

Clinical data:
In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% “Verdox ®” in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15)

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (58).

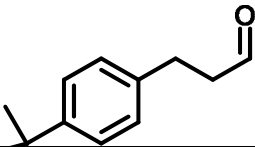
4-TERT-BUTYLCYCLOHEXYL ACETATE	
CAS # 32210-23-4	
EC # 250-954-9	
4-(1,1-Dimethylethyl)cyclohexyl acetate	
Boisinal A 464D; Cyclohexanol, 4-tert-Butyl-, acetate; Cyclohexanol, 4-(1,1-Dimethylethyl)-, acetate; 4-(1,1-Dimethylethyl)cyclohexyl acetate; 4-tert-Butylcyclohexanol acetate; Dorisyl; Madeflor; NSC 163103; Oryclone, Oryclone special, Oryclon extra; p-t-BCHA; p-tert-Butylcyclohexyl acetate; para-tert-Butylcyclohexyl acetate; PTBCHA; Velvetone; Verbeniax; Vertenex; Vertinate; Vertopol; Ylanate	

Current regulation: /

Clinical data:
In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% “Vertenex ®” in pet., tested in 107 consecutive patients in High Wycombe, were observed (15).

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (59).

p-tert -Butyldihydrocinnamaldehyde	
CAS # 18127-01-0	
EC # 242-016-2	

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

Current regulation: III/155

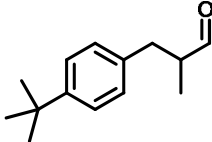
Clinical data: /

Additional

information:

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

http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=39132

BUTYLPHENYL METHYLPROPIONAL (Lilial®)	
CAS # 80-54-6	
EC # 201-289-8	
3-(4-tert-Butylphenyl)-2-methylpropanal	
p-t-Butyl- α -methylhydrocinnamic aldehyde; 2-(4-tert-Butylbenzyl)propionaldehyde (REACH, EINECS); 4-(1,1-Dimethylethyl)- α -methyl-benzenepropanal; Hydrocinnamaldehyde, p-tert-Butyl- α -methyl-; ($\pm$)-2-Methyl-3-(4-tert-butylphenyl)propanal; 2-Methyl-3-(4-tert-butylphenyl)propanal; 2-[(4-tert-Butylphenyl)methyl]propanal; 3-(4-tert-Butylphenyl)-2-methylpropanal; 3-(p-tert-Butylphenyl)-2-methylpropionaldehyde; 3-(p-tert-Butylphenyl)isobutylaldehyde; 4-(1,1-Dimethylethyl)- α -methylbenzenepropanal; 4-tert-Butyl- α -methylhydrocinnamic aldehyde; Lilestralis; Lilial; Lysmeral; NSC 22275; lilestral; p-tert-Butyl- α -methylhydrocinnamaldehyde; p-tert-Butyl- α -methylhydrocinnamic aldehyde; pt-Bucinal; α -Methyl-p-tert-butylhydrocinnamaldehyde; β -Lilial	

Current regulation: Annex III, part 1, n° 83

Clinical data:

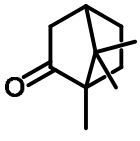
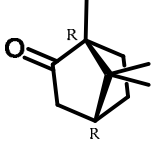
In the “background information” section of the 1999 opinion, lilial is classified as “less frequently reported allergen”; with 2 cases of contact allergy reported in 1 study of 176 eczema patients and 1 case with contact allergy to Lilial from a deodorant; a number of other reported positive cases were considered to possibly have been false positive (33).

Since the last SCCNFP-opinion of 1999, the Frosch 2002a study yielded 0.2% positive reactions to Lilial® (10% pet.) among the 1855 consecutive patients tested (16). The IVDK 2007 study yielded 0.4% (95% CI: 0.2 – 0.8%) positive reactions in 2004 patients consecutively tested (4). The IVDK 2010 study, 0.62% (95% CI: 0.04 – 1.21%; percentages standardised for age and sex) of 1947 patients PTed reacted to the compound (7). In the Groningen 2009 study, n=2, i.e. 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen, tested at only 1% pet. (6). In the deGroot 2000 study, 9 of 1825 consecutively tested patients had a positive reaction to lilial® (5%

pet.) (12). Lilial® has been identified as constituent of perfumes used by a patient, causing ACD (60).

Additional information:

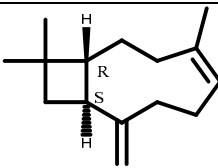
It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010).

CAMPHOR	 76-22-2
CAS # 76-22-2 / 464-49-3	
EC # 207-355-2 / 200-945-0	
1,7,7-Trimethyl-bicyclo[2.2.1]heptan-2-one (76-22-2) (1R,4R)-1,7,7-Trimethyl-bicyclo[2.2.1]heptan-2-one (464-49-3)	
76-22-2: DL-Bornan-2-one (REACH, EINECS); 2-Bornanone; Bornan-2-one, INCI name according to CAS; CAMPHOR/DL-bornan-2-one; Camphor; (±)-Camphor; DL-Camphor; 1,7,7-Trimethylnorcamphor; 2-Camphanone; Alphanon; Borneo camphor; Root bark oil; Spirit of camphor 464-49-3: (1R)-1,7,7-Trimethyl-bicyclo[2.2.1]heptan-2-one; (1R,4R)-(+)-Camphor; (+)-2-Bornanone; (+)-Camphor; (1R)-(+)-Camphor; (1R)-Camphor; (1R,4R)-(+)-Camphor; (R)-(+)-Camphor; (R)-Camphor; Camphor; D-Camphor; D-(+)-Camphor; Alcanfor; Japanese camphor.	 464-49-3

Current regulation: /

Clinical data:
From the UK, a case of allergic contact dermatitis after application of Earex ® ear drops due to rectified camphor oil (tested 10% pet.) was reported (61). Application of a liquid rubefacient of Asian origin caused allergic contact dermatitis in a 58-year-old patient, according to the positive PT result with 10% camphor ("alcaonfor") in pet. due to this ingredient (62). In the US, a case of contact dermatitis due to "Vics VapoRub" has been reported (63).

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

beta-CARYOPHYLLENE	
CAS # 87-44-5	
EC # 201-746-1	
(1R,4E,9S)-4,11,11-Trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene	
(E)-(1R,9S)-(-)-4,11,11-Trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene; [1R-(1R*,4E,9S*)]-4,11,11-Trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene; (-)-(E)-Caryophyllene; (-)-Caryophyllene; (-)-E-Caryophyllene; (-)-trans-Caryophyllene; (-)-β-Caryophyllene; (E)-Caryophyllene; Caryophyllene; Caryophyllene B; NSC 11906; l-Caryophyllene; trans-Caryophyllene; β-Caryophyllen; β-Caryophyllene; (-)-β-Caryophyllene	

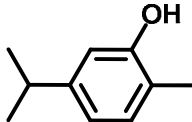
Current regulation: /

Clinical data:

In the Frosch 2002 b study, 0.6% positive reactions to caryophyllene (5% pet.) in 1606 consecutive were observed (17).

Additional information:

beta-Caryophyllene autoxidizes at air exposure. As the primary oxidation products, the hydroperoxides, are very unstable and immediately form epoxides with low sensitizing capacity, the increase in allergenic activity caused by autoxidation is comparably low (64). A multicenter study identified 0.5% positive reactions to oxidized *beta*-caryophyllene (3.0% pet.) in 1511 consecutive patients (65). Of these, 2 patients (0.1%) reacted to the major oxidation product (caryophyllene oxide) (3.9% pet.).

CARVACROL	
CAS # 499-75-2	
EC # 207-889-6	
2-Methyl-5-(1-methylethyl)-phenol	
2-Hydroxy-1-methyl-4-(1-methylethyl)benzene; 2-Hydroxy-p-cymene; 2-Methyl-5-(1-methylethyl)phenol; 2-Methyl-5-isopropylphenol; 3-Isopropyl-6-methylphenol; 5-Isopropyl-2-methylphenol; 5-Isopropyl-o-cresol; 6-Methyl-3-isopropylphenol; Antioxine; Dentol; Isopropyl o-cresol; Isothymol; NSC 6188; p-Cymen-2-ol	

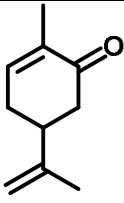
Current regulation: /

Clinical data:

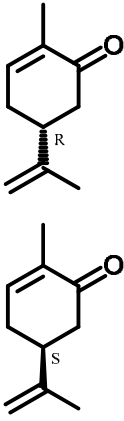
The DeGroot 1985 study identified 2 (1.1%) positive reactions among 179 patients using a 5% PT preparation of this compound – these reactions may have been at least partly due to an “excited back syndrome” and are thus of limited evidence (25). Meynadier et al. ¹¹ patch tested 28 patients with contact allergy to fragrance ingredients using 2% carvacrol in pet. Positive reactions were observed in 3 of 28 patients (after (66)).

Additional information:

Carvacrol is derived from p-cymene by sulfonation followed by alkali fusion. Carvacrol can also be derived from savory, thyme, marjoram, oregano, lovage root, and Spanish origanum oil (66). Carvacrol is a flavor ingredient that can be found in alcoholic beverages, baked goods, chewing gum, condiment relish, frozen dairy, gelatin pudding, non-alcoholic beverages, and soft candy at concentrations from 0.1 to 28.54 ppm (RIFM 2001, according to (66)).

CARVONE	
CAS # 99-49-0 / 6485-40-1 / 2244-16-8	
EC # 202-759-5 / 229-352-5 / 218-827-2	
2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-one (99-49-0)	

¹¹ Meynadier, J. M., J. Meynadier, J. L. Peyron, and L. Peyron. 1986. Clinical forms of skin manifestations in allergy to perfume. *Ann. Dermatol. Venerol.* 113:31–39.

(5R)-2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-one (6485-40-1) (5S)-2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-one (2244-16-8)	
99-49-0: p-Mentha-6,8-dien-2-one; (±)-Carvone; 2-Methyl-5-isopropenyl-2-cyclohexenone; 5-Isopropyl-2-methyl-2-cyclohexen-1-one; Carvone; DL-Carvone; Karvon; Limonen-6-one; NSC 6275; p-Mentha-1(6),8-dien-2-one 6485-40-1: R)-(-)-p-Mentha-6,8-dien-2-on); (-)-(5R)-Carvone; (-)-(R)-Carvone; (-)-Carvone; (-)-p-Mentha-6,8-dien-2-one; (4R)-(-)-Carvone; (R)-(-)-Carvone; (R)-Carvone; L-(-)-Carvone; L-Carvone; l-1-Methyl-4-isopropenyl-6-cyclohexen-2-one; l-Carvone 2244-16-8: (S)-(+)-p-Mentha-6,8-dien-2-one; (+)-Carvone; (S)-(+)-Carvone; (S)-(+)-p-Mentha-6,8-dien-2-one; (S)-Carvone; (+)-Carvone; D-(+)-Carvone; D-Carvone; Talent; d-1-Methyl-4-isopropenyl-6-cyclohexen-2-one; (S)-2-Methyl-5-(1-methylvinyl)cyclohex-2-en-1-one; d-Carvone	

Current regulation: /

Clinical data:

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

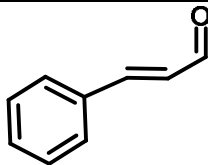
“Carvone has occasionally been reported as an allergen, usually in flavourings. Isomers of carvone have been either a mint or a rye flavour and aroma. We report a woman with positive patch-test reactions to carvone (newly added to the North American Contact Dermatitis Group standard series) and dermatitis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

Additional information:

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

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

¹² <http://www.ncbi.nlm.nih.gov/pubmed/20233552>

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

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

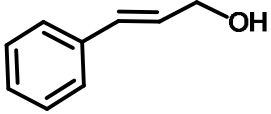
Clinical data:
In the “background information” section of the previous opinion (33), cinnamal, one of the 8 constituents of the FM I, is classified as frequent allergen, causing allergic reactions in a notable persons with eczema from cosmetic products in several studies (33).

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

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

Additional information:

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

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

Opinion on fragrance allergens in cosmetic products

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

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

Clinical data:

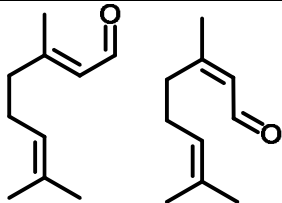
In the “background information” section of the previous opinion (33), cinnamyl alcohol, one of the 8 constituents of the FM I, is classified as frequent allergen, causing allergic reactions in a notable persons with eczema from cosmetic products (33).

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

Additional information:

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

A RIFM review is available (81)

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

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

Clinical data:

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

cosmetic products (33).

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

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

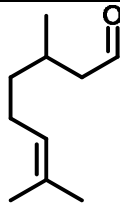
Additional information:

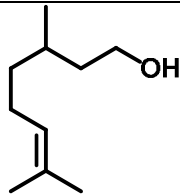
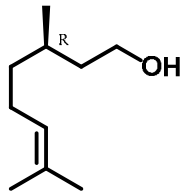
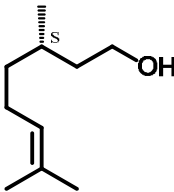
Citral is the mixture of two isomers: cis-citral (neral) and trans-citral (geranial).

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

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

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

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

CITRONELLOL			
CAS # 106-22-9 / 1117-61-9 / 7540-51-4			
EC # 247-737-6 / 214-250-5 / 231-415-7			
3,7-Dimethyl-6-octen-1-ol (106-22-9); (3R)-3,7-Dimethyl-6-octen-1-ol (1117-61-9); (3S)-3,7-Dimethyl-6-octen-1-ol (7540-51-4)			106-22-9
106-22-9: (±)-3,7-Dimethyl-6-octen-1-ol; (±)-Citronellol; (±)-β-Citronellol; 2,3-Dihydrogeraniol; 2,6-Dimethyl-2-octen-8-ol; Cephrol; Citronellol; Citronello 950; DL-Citronellol; Dihydrogeraniol; NSC 8779; Rodinol; dl-Citronellol; β-Citronellol			
1117-61-9: (R)-3,7-Dimethyl-6-octen-1-ol; (R)-(+)-3,7-Dimethyl-6-octen-1-ol; (+)-(R)-Citronellol; (+)-Citronellol; (+)-β-Citronellol; (3R)-(+)-β-Citronellol; (R)-(+)-Citronellol; (R)-(+)-β-Citronellol; (R)-Citronellol; (R)-β-Citronellol; D-Citronellol; d-Citronellol	1117-61-9	7540-51-4	
7540-51-4: (-)-3,7-Dimethyl-6-octen-1-ol; (-)-(S)-Citronellol; (-)-Citronellol; (-)-β-Citronellol; (S)-(-)-Citronellol; (S)-(-)-β-Citronellol; (S)-3,7-Dimethyl-6-octen-1-ol; (S)-Citronellol; (S)-β-Citronellol; L-Citronellol; l-Citronellol			

Current regulation: Annex III, part 1, n° 86

Clinical data:

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

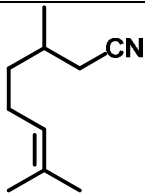
Since the last SCCNFP-opinion of 1999, in the Larsen 2002 c study, „DL citronello“ (5% in pet.) elicited positive PT reactions in 8.7% of the patients (1). In 1855 consecutive patients of the Frosch 2002 a study, 0.4% positive reactions were noted (16). In the EU 2005 study, 4 of 1701 patients (0.2%,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

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

Additional information:

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

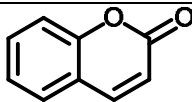
RIFM reviews have been published regarding L-citronellol (86), D-citronellol (87) and DL-citronellol (88). Another review is available by Hostynek and Maibach (89). It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010).

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

Current regulation: /

Clinical data: /

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

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

Current regulation: Annex III, part1, n° 77

Clinical data:

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

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

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

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

Additional information:

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

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

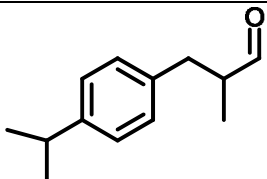
Coumarin is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010).

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

Current regulation: /

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

Additional information: ...

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

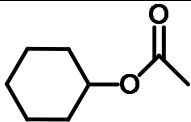
methylpropionaldehyde; methylpropionaldehyde(REACH, EINECS); methylhydrocinnamic aldehyde; Cyclamal; Cyclamen aldehyde; Cyclosal; Cyclosal perfume; Cymal; p-Isopropyl- α - methylhydrocinnamaldehyde; methylethyl)benzenepropanal; isopropylhydrocinnamaldehyde	3-p-Cumenyl-2- 4-Isopropyl- α - α -Methyl-4-(1-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

Current regulation: ...

Clinical
/

data:

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

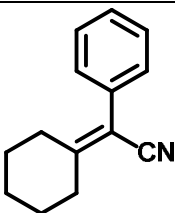
CYCLOHEXYL ACETATE	
CAS # 622-45-7	
EC # 210-736-6	
Cyclohexyletanoat	
Acetic acid cyclohexanyl ester; Acetoxycyclohexane; Cyclohexyl acetate; NSC 8772	

Current regulation: /

Clinical data:

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

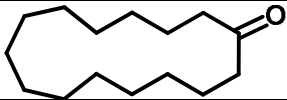
Additional information: A RIFM review is available (92).

<i>alpha</i>-CYCLOHEXYLIDENE BENZENEACETONITRILE	
CAS # 10461-98-0	
EC # 423-740-1	
α-Cyclohexylidenebenzeneacetonitrile	
alpha-Cyclohexylidene-benzeneacetonitrile (REACH); Δ 1 α -Phenyl- α -Cyclohexaneacetonitrile; 2-Cyclohexylidene-2-phenylacetonitrile; NSC 408284; Peonile (REACH)	

Current regulation: /

Clinical data: /

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

CYCLOPENTADECANONE	
CAS # 502-72-7	

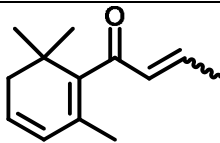
Opinion on fragrance allergens in cosmetic products

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

Current regulation: /

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

Additional information: ...

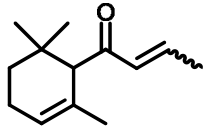
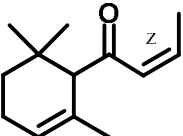
DAMASCENONE	
ROSE KETONE-4 (Not officially an INCI Name but Perfuming Name; Damascenone as such is not listed in CosIng)	
CAS # 23696-85-7	
EC # 245-833-2	
1-(2,6,6-Trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one	
1-(2,6,6-Trimethyl-1,3-cyclohexadienyl)-2-buten-1-one; 1-Crotonoyl-2,6,6-trimethyl-1,3-cyclohexadiene; 2,6,6-Trimethyl-1-(2-butenoyl)-1,3-cyclohexadiene; 2,6,6-Trimethyl-1-crotonyl-1,3-cyclohexadiene; Rose ketone # 4	

Current regulation: Annex III, part1, n° 160 (max. conc. 0.02%)

Clinical data: /

Additional information:

RIFM reviews are available (93, 94), quoting 1 negative, and 2 positive (2 of 37, 1 of 50 volunteers) HRIPTs with damascenone based on 2 LLNA, the EC3 values were calculated as 1.24% and 1.22%, respectively (94).

<i>alpha-DAMASCONE (TMCHB)</i>	 43052-87-5
CAS # 43052-87-5 / 23726-94-5	
EC # x / 245-845-8	
1-(2,6,6-Trimethylcyclohex-2-en-1-yl)but-2-enone (43052-87-5); (2Z)-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one (23726-94-5)	
43052-87-5: 2,6,6-Trimethyl-1-crotonyl-2-cyclohexene; α -Damascone	 23726-94-5
23726-94-5: (Z)-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one; (Z)- α -Damascone; cis- α -Damascone	

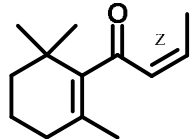
Current regulation: Annex III, part1, n° 157 (max. conc. 0.02%)

Clinical data:
In the Frosch 2002 b study, n=8 (0.5%) mostly strong positive PT reactions to consecutive patients were noted using a mixture of alpha and beta damascene, 0.1% pet. each (17). In human sensitisation experiments, after epicutaneous induction with 30% 1-(2,6,6-trimethylcyclohex-2-en-1-yl)but-2-enone (TMCHB, CAS # 43052-87-5) with adjuvant, to enhance response to this weak sensitiser, 8 of 30 patients were elicited by a challenge with 3% TMCHB 2 weeks later (95).

Additional information:

The former CAS # refers to alpha-Damascone or 1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-Buten-1-one. The latter CAS # refers to the identified ingredient cis-alpha-Damascone or (Z)-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one, the content of which is restricted (SCCS-opinion 0392/00).

A RIFM review is available on alpha-damascone (96), quoting a number of partly positive HRIPT and other human studies, as well as different animal experiments. In 1 LLNA reported, an EC3 value of 3.3% was found. Another RIFM review is available for cis-alpha-damascone (97), supplying, however, no data on sensitisation.

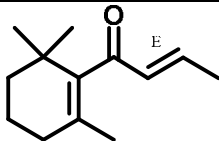
<i>cis-beta-DAMASCONE</i>	
CAS # 23726-92-3	
EC # 245-843-7	
(2Z)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one	
(Z)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one; (Z)- β -Damascone	

Current regulation: Annex III, part 1, n° 162 (max. conc. 0.02%)

Clinical data:
Regarding results of the Frosch 2002 b study, see under alpha-damascone.

Additional information:

A RIFM review is available (98), citing several negative and one positive HRIPTs, and a number of – mostly positive – animal experiments.

<i>trans-beta-DAMASCONE</i>	
CAS # 23726-91-2	
EC # 245-842-1	
(2E)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one	
(E)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one; (E)-β-Damascone; Damascone beta; trans-2,6,6-Trimethyl-1-crotonylcyclohex-1-ene; trans-β-Damascone; β-Damascone	

Current regulation: Annex III, part 1, n° 158 (max. conc. 0.02%)

Clinical data: /

Additional information:

A RIFM review is available (99), citing 2 negative HRIPT and 1 negative maximisation test, and a number of positive animal experiments (the EC3 value, based on 1 LLNA, was found to be 2.4%).

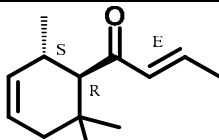
<i>delta-DAMASCONE</i>	
CAS # 57378-68-4	
EC # 260-709-8	
1-(2,6,6-Trimethyl-3-cyclohexen-1-yl)-2-buten-1-one	
δ-Damascone	

Current regulation: Annex III, part 1, n° 161 (max. conc. 0.02%)

Clinical data: /

Additional information:

A RIFM review is available (100), citing several positive HRIPT and 1 negative HRIPT. Cross sensitisation to alpha- and beta-damascone was demonstrated in 3 sensitised subjects. 2 LLNA studies are reported on, yielding EC3 values of 5.19% and 9.6%, resp.

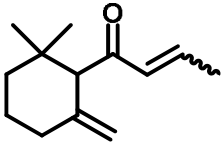
<i>trans-trans-delta-DAMASCONE</i>	
CAS # 71048-82-3	
EC # 275-156-8	
(2E)-rel-1-[(1R,2S)-2,6,6-Trimethyl-3-cyclohexen-1-yl]- 2-buten-1-one	
[1α(E),2β]-1-(2,6,6-Trimethyl-3-cyclohexen-1-yl)-2-buten-1-one; trans-δ-Damascone; δ-Damascone; trans, trans-δ-Damascone	

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%).

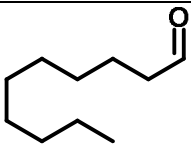
<i>gamma-DAMASCONE</i>	
CAS # 35087-49-1	
EC # 481-910-9	
1-(2,2-Dimethyl-6-methylenecyclohexyl)-2-buten-1-one	
γ-Damascone	

Current regulation: /

Clinical data: /

Additional information:

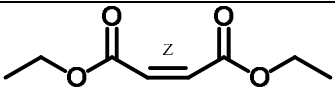
A RIFM review is available (102), citing 1 positive Buehler test and 1 LLNA study yielding an EC3 value of 4.6%

<i>DECANAL</i>	
CAS # 112-31-2	
EC # 203-957-4	
n-Decanal	
Capraldehyde; Capric aldehyde; Caprinaldehyde; Caprinic aldehyde; Decaldehyde; Decanaldehyde; Decyl aldehyde; Decylic aldehyde; NSC 6087; n-Decaldehyde; n-Decyl aldehyde	

Current regulation: /

Clinical data: /

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

<i>DIETHYL MALEATE</i>	
CAS # 141-05-9	
EC # 205-451-9	
(2Z)-Diethyl but-2-enedioate	
2-Butenedioic acid (2Z)-, diethyl ester; 2-Butenedioic acid (Z)-, diethyl ester; Maleic acid, diethyl ester; (2Z)-2-Butenedioic acid diethyl ester; Diethyl (Z)-2-butenedioate; Ethyl maleate; Staflex DEM	

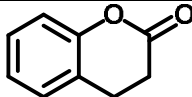
Current regulation: Annex II, n° 426

Clinical

data:

In the Malten 1984 study, 3.2% of 182 patients displayed a positive PT reaction to diethyl maleate 0.1% pet. (24). In this study, it has been noted that “in the max. test and clinically this is a strong sensitiser having caused patch test sensitisation (42%)”

Additional information: /

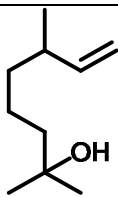
DIHYDROCOUMARIN	
CAS # 119-84-6	
EC # 204-354-9	
3,4-Dihydro-2H-1-benzopyran-2-one	
Hydrocoumarin; Hydrocinnamic acid, o-hydroxy-, δ -lactone; 2-Chromanone; 3,4-Dihydro-1H-benzopyran-2-one; 3,4-Dihydrocoumarin; Dihydrocoumarin; Melilotin; Melilotin (coumarin); Melilotol	

Current regulation: Annex II, n° 427

Clinical data:

In the Malten 1984 study, 3.7% of 182 patients displayed a positive PT reaction to dihydrocoumarine 5% pet. (24).

Additional information: /

DIHYDROMYRCENOL	
CAS # 18479-58-8	
EC # 242-362-4	
(±)-2,6-Dimethyloct-7-en-2-ol	
1,1,5-Trimethyl-6-heptenol; 2,6-Dimethyl-7-octen-2-ol; 3,7-Dimethyl-1-octen-7-ol; 2,6-Dimethyl-7-octen-2-ol (INCI)	

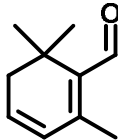
Current regulation: /

Clinical data: /

Additional information:

A RIFM review is available (103), listing 2 negative HRIPTs and 1 negative human maximisation test.

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

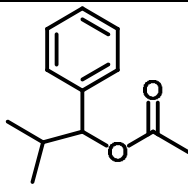
2,3-DIHYDRO-2,2,6-TRIMETHYLBENZALDEHYDE	
CAS # 116-26-7	
EC # 204-133-7	
2,6,6-Trimethyl-1,3-cyclohexadiene-1-carboxaldehyde	
2,2,6-Trimethyl-4,6-cyclohexadien-1-aldehyde; 2,6,6-Trimethyl-1,3-cyclohexadiene-1-aldehyde; Safranal	

Current regulation: /

Clinical data: /

Additional information:

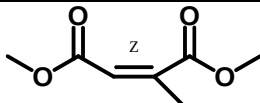
A RIFM review quotes one positive HRIPT (5 of 53) and one negative HRIPT (0 of 54) (93).

DIMETHYLBENZYL CARBINYL ACETATE (DMBCA)	
CAS # 151-05-3	
EC # 205-781-3	
2-Methyl-1-phenylpropyl acetate	
Benzeneethanol, α,α-dimethyl-, acetate; Phenethyl alcohol, α,α-dimethyl-, acetate; 1,1-Dimethyl-2-phenylethyl acetate; 2-Methyl-1-phenyl-2-propyl acetate; 2-Methyl-1-phenylpropan-2-yl acetate; Benzyl dimethylcarbinol acetate; Benzyl dimethylcarbinyl acetate; Dimethylbenzylcarbinol acetate; Dimethylbenzylcarbonyl acetate; NSC 46123; α,α-Dimethylphenethyl acetate	

Current regulation: /

Clinical data:
In the Frosch 2002 a study, 0.2% positive PT reactions to consecutive patients were noted (16). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and one to 5% DMBCA in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15).

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

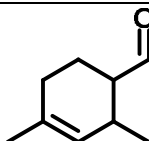
<i>DIMETHYL CITRACONATE</i>	
CAS # 617-54-9	
EC #	
(2Z)-Diethyl-2-metyl-but-2-enedioate	
(2Z)-2-methyl-2-Butenedioic acid, dimethyl ester; 2-Butenedioic acid, 2-methyl-, dimethyl ester, (Z)-; Citraconic acid, dimethyl ester; Dimethyl methylmaleate; Methylmaleic acid, dimethyl ester	

Current regulation: Annex II, n° 431

Clinical data:

In the Malten 1984 study, 3.7% of 182 patients displayed a positive PT reaction to dimethylcitraconate 12% pet. (24). In this paper, a human maximisation test positive in “4/44” is quoted.

Additional information: ...

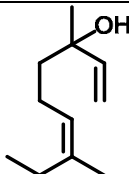
2,4-DIMETHYL-3-CYCLOHEXEN-1-CARBOXALDEHYDE	
CAS # 68039-49-6	
EC # 268-264-1	
2,4-Dimethyl-cyclohex-3-ene-1-carboxaldehyde	
(Z)-Vertocitral C; 2,4-Dimethyl-3-cyclohexene-1-carboxaldehyde; 2,4-Dimethyl-3-cyclohexenecarboxaldehyde; 2,4-Dimethyl-3-cyclohexenylcarbaldehyde; Cyclal C; Ligustral; Tricyclal; Triplal; Tripral; Zestover	

Current regulation: /

Clinical data: /

Additional information:

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

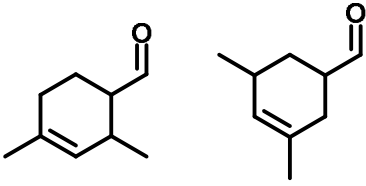
3,7-DIMETHYL-1,6-NONADIEN-3-OL	
CAS # 10339-55-6	
EC # 233-732-6	
(7Z)-3,7-Dimethyl-1,6-nonadien-3-ol	
Ethyl linalool; Methyllinalool	

Current regulation: /

Clinical data: /

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (104), citing 1 negative human maximisation test (n=25).

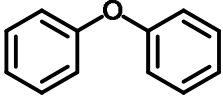
DIMETHYLTETRAHYDRO BENZALDEHYDE	
CAS # 68737-61-1	
EC # 272-113-5	
2,4-Dimethyl-cyclohex-3-ene-1-carboxaldehyde 3,5-Dimethyl-cyclohex-3-ene-1-carboxaldehyde	
Hivertal; Vertocitral	

Current regulation: /

Clinical data:

In the Larsen 2001 study, 2.3% positive PT reactions were observed with the isomer mixture, tested 5% pet., in 178 patients with known contact allergy to fragrance ingredients (19).

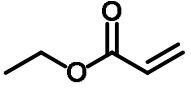
Additional information: /

DIPHENYL ETHER	
CAS # 101-84-8	
EC # 202-981-2	
Phenyl ether	
1,1'-oxybis-Benzene; Barrel Therm 330; Benzene, phenoxy-; Biphenyl oxide; Chemcryn JK-EB; Diphenyl ether; Diphenyl oxide; NSC 19311; Oxybisbenzene; Phenoxybenzene; Phenyl oxide	

Current regulation: /

Clinical data: /

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

ETHYL ACRYLATE	
CAS # 140-88-5	
EC # 205-438-8	
Ethyl 2-propenoate	
Acrylic acid ethyl ester (6CI,8CI); 2-Propenoic acid ethyl ester; Ethyl 2-propenoate; Ethyl acrylate; Ethyl acrylic ester; Ethyl propenoate; NSC 8263	

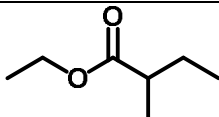
Current regulation: Annex II, n° 435

Clinical data:

In the Malten 1984 study, n=1 (0.5%) of 182 patients displayed a positive PT reaction to ethyl acrylate 1% pet. (24). In the NACDG 2009 multicentre study, 0.9% of

consecutive patients (n=4428) had a positive PT reaction (21).

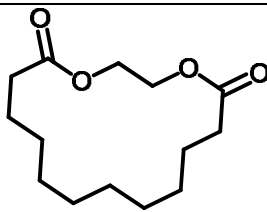
Additional information: /

ETHYL 2-METHYLBUTYRATE	
CAS # 7452-79-1	
EC # 231-225-4	
Ethyl 2-methylbutyrate	
Butyric acid, 2-methyl-, ethyl ester (6CI,7CI,8CI); (±)-Ethyl 2-methylbutanoate; 2-Methylbutanoic acid ethyl ester; 2-Methylbutyric acid ethyl ester; Ethyl 2-methylbutanoate; Ethyl 2-methylbutyrate; Ethyl α-methylbutyrate; NSC 1103	

Current regulation: /

Clinical data: /

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

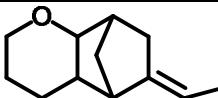
ETHYLENE DODECANEDIOATE	
CAS # 54982-83-1	
EC # 259-423-6	
1,4-Dioxacyclohexadecane-5,16-dione	
Cyclic ethylene dodecanedioate; Ethylene dodecanedioate; Musk 144; Musk C-14	

Current regulation: /

Clinical data:

In the Larsen 2002 c study on 218 patients with known contact allergy to fragrance ingredients, this compound caused 0.9% positive PT reactions at 5% pet. (1).

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

6-ETHYLIDENEOCTAHYDRO-5,8-METHANO-2H-BENZO-1-PYRAN	
CAS # 93939-86-7	
EC # 300-376-9	
6-Ethylideneoctahydro-5,8-methano-2H-1-benzopyran	

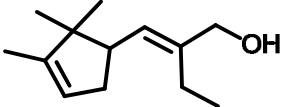
Current regulation: /

Clinical

data:

In the Larsen 2001 study, no positive PT reactions were observed with this compound, tested 5% pet., in 178 patients with known contact allergy to fragrance ingredients (19).

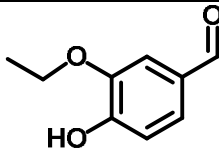
Additional information: /

2-ETHYL-4-(2,2,3-TRIMETHYL-3-CYCLOPENTEN-1-YL)-2-BUTEN-1-OL	
CAS # 28219-61-6	
EC # 248-908-8	
2-Ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)-2-buten-1-ol	
2-Ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)-2-buten-1-ol; 2-Ethyl-4-(2',2',3-trimethylcyclopent-3'-enyl)but-2-enol; Bacdanol; Bangalol; Dartanol; Finanol; Levosandol; Radjanol; Sanjinol	

Current regulation: /

Clinical data: /

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

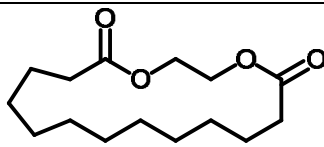
ETHYL VANILLIN	
CAS # 121-32-4	
EC # 204-464-7	
3-Ethoxy-4-hydroxybenzaldehyde	
2-Ethoxy-4-formylphenol; 3-Ethoxy-4-hydroxybenzaldehyde; 3-Ethylvanillin; 4-Hydroxy-3-ethoxybenzaldehyde; Arovanillon; Bourbonal; Ethavan; Ethovan; Ethylprotal; Ethylvanillin; NSC 1803; NSC 67240; Protocatechuic aldehyde 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).

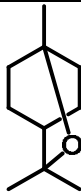
Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

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	

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6171; Terpan; p-Cineole	

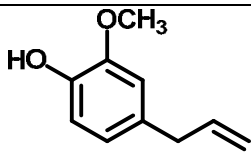
Current regulation: /

Clinical data: /

Additional information:

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

See also ***EUCALYPTUS SPP. LEAF OIL***; eucalyptol is the major ingredient there (up to 85%), but found in significant quantities also in a number of other essential oils (see 3.2).

EUGENOL	
CAS # 97-53-0	
EC # 202-589-1	
2-Methoxy-4-(2-propen-1-yl)-phenol	
Other names: 4-Allyl-2-methoxy-phenol; 1-Allyl-4-hydroxy-3-methoxybenzene; 2-Hydroxy-5-allylanisole; 2-Methoxy-1-hydroxy-4-allylbenzene; 2-Methoxy-4-(2-propenyl)phenol; 2-Methoxy-4-(2'-propenyl)phenol; 2-Methoxy-4-[2-allyl]phenol; 2-Methoxy-4-allylphenol; 3-(3-Methoxy-4-hydroxyphenyl)propene; 3-(4-Hydroxy-3-methoxyphenyl)-1-propene; 4-Allyl-1-hydroxy-2-methoxybenzene; 4-Allyl-2-methoxyphenol; 4-Allylguaiacol; 4-Hydroxy-3-methoxyallylbenzene; Allylguaiacol; Bioxeda; Caryophyllic acid; Dentogum; Eugenig acid; Eugenol; NSC 209525; NSC 8895; p-Allylguaiacol; p-Eugenol	

Current regulation: Annex III, part 1, n° 71

Clinical data:

In the “background information” section of the previous opinion (33), eugenol, one of the 8 components of the FM I, is classified as frequent allergen, causing allergic reactions in about 1.2% in consecutive PT patients and accounting for 4 to 16% of reactions to the FM I. Allergic reactions had been observed in 0.7 – 20% of patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.5% (95% CI: 0.3 – 1.0%) positive reactions in 2065 consecutively PTed patients (4). In the Groningen 2009 study, 1.3% (95% CI: 0.3 – 3.2%) had positive reactions to eugenol, tested at 2% pet., i.e., twice the commonly used concentration (6). F. Giusti et al. examined 1754 consecutive patients tested with eugenol 1% pet. in addition to the baseline series, 09/1998 - 01/2000. 21 patients (1.2%) reacted positively to eugenol (106). In the An 2005 study, 8 of 422 consecutive patients, i.e., 1.9%, had positive reaction (13) (test concentration 2%). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 2.5% positive reactions (22). The IVDK 2010 study, 0.44% (95% CI: 0.04 – 0.84%) of 1214 consecutively tested patients reacted to the compound, while 1.57% (95% CI: 1.19 – 1.95%) of 4801 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=12 reacted positively to eugenol (48).

Moreover, eugenol is capable of inducing immediate type reactions of the airways, as illustrated by the well-documented case of a 30 year old hairdresser who developed severe occupational bronchial asthma due to eugenol (107). A case of urticaria after dental treatment with eugenol-containing material was reported from India (108); however, occasional cases are also reported from Europe (109). Occupational exposure to eugenol / zinc oxide type dental restorative material, which is apparently less frequently used nowadays, may lead to occupational sensitisation to eugenol, as illustrated by a case report (110).

Additional information:

Eugenol is the main component (80-95%) of clove oil, but also found in citronella oil, pimento leaf oil and cinnamon bark oil (see section 3.2).

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

FARNESOL

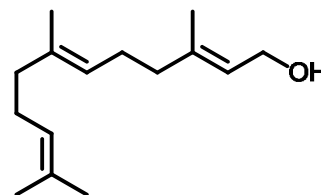
CAS # 4602-84-0

EC # 225-004-1

3,7,11-Trimethyl-2,6,10-Dodecatrien-1-ol

Farnesol; 3,7,11-Trimethyl-2,6,10-dodecen-1-ol; FCI 119a; Farnesyl alcohol; NSC 60597; Nikkosome

Current regulation: Annex III, part 1, n° 82



Clinical data:

In the “background information” section of the 1999 opinion, farnesol is classified as “less frequently reported allergen”; in 1 study of patients with cosmetic dermatitis 2 cases with contact allergy to farnesol had been reported; in other studies, positive reactions were seen in patients with positive PT reactions to MPR (33).

Since the last SCCNFP-opinion of 1999, farnesol is used not only for its scent, but also for its (slight) antimicrobial activity, useful, for instance, in deodorants. Thus, axillary dermatitis is a relatively typical presentation (111). In a multicentre study based on 1997/98 PT data, 0.5% positive reactions in consecutive patients were noted (Frosch 2002 a (16)). Farnesol is included in the FM II. In the original publication on single constituents of the FM II, 6 of 1701 consecutive patients reacted positively to farnesol 5%, ie., 0.35% (95% CI: 0.13 – 0.77%) (10). In a study on consecutive patients tested in 2003, 38 of 4238 patients had positive reactions to farnesol 5% pet. (0.9%, 95% CI: 0.6 – 1.2%) (4)(IVDK 2007). (A paper on farnesol previously published by the IVDK (112) presents results included in this later analysis.) In a series from Nagoya, Japan, 1.1% positive reactions in 1483 patients with suspected cosmetic dermatitis were observed (tested at 5% pet.) (14). In the Groningen 2009 study, 0.9% (95% CI: 0.2 – 2.7%) had positive reactions (6).

Additional information:

“Farnesol is an acyclic primary sesquiterpene alcohol found in essential oils such as lemongrass, citronella, tuberose blossom, sandalwood and orange blossom” (23). A RIFM review is available (113).

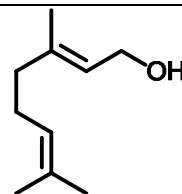
GERANIOL

CAS # 106-24-1

EC # 203-377-1

(2E)-3,7-Dimethyl-2,6-octadien-1-ol

(E)-3,7-Dimethyl-2,6-octadien-1-ol; (E)-Geraniol; (E)-Nerol; 3,7-Dimethyl-trans-2,6-octadien-1-ol; Geraniol; Geranyl alcohol; Lemonol; MosquitoSafe; NSC 9279; trans-3,7-Dimethyl-2,6-octadien-1-ol; trans-Geraniol; β-Geraniol



Current regulation: Annex III, part 1, n° 78

Clinical data:

In the “background information” section of the previous opinion (33), geraniol, one of the 8 components of the FM I, is classified as frequent allergen, causing allergic reactions in about 0.4% in consecutive PT patients and accounting for 3 to 7% of reactions to the FM I. Allergic reactions had been observed in 1.2 – 30% of patients with

eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.5% (95% CI: 0.2 – 0.9%) positive reactions in 2063 consecutively PTed patients (4). In the Groningen 2009 study, 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen, tested at 2%, i.e. twice the usual concentration (6). In a series from Nagoya, Japan, 0.3% positive reactions in 1483 patients with suspected cosmetic dermatitis were observed (tested at the unusually high concentration of 5% pet.) (14). In the Wöhrle 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).

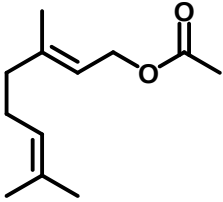
The fact that geraniol also occurs in food flavourings, and can elicit signs and symptoms of manifest contact sensitisation, is illustrated by the case of a 19 year old Japanese woman with cheilitis due to geraniol, improving after avoidance of respective foodstuff (114). A 20 year old Japanese woman 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 autooxidation at air exposure and via metabolic oxidation. Geraniol and neral together with hydroperoxide have been identified as oxidation products when geraniol autooxidizes (84). Geraniol and neral were also identified as metabolites of geraniol (85). This explains the simultaneous reactions to geraniol and citral seen by (4).

A review is available by Hostynek and Maibach (116) and by RIFM (117). It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010).

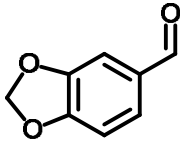
GERANYL ACETATE	
CAS # 105-87-3	
EC # 203-341-5	
(2E)-1-Acetate-3,7-dimethyl-2,6-octadien-1-ol	
(E)-Acetat-3,7-dimethyl-2,6-Octadien-1-ol; Geraniol acetate; (E)-3,7-Dimethyl-2,6-octadien-1-ol acetate; (E)-3,7-Dimethyl-2,6-octadienyl acetate; Acetic acid (2E)-3,7-dimethyl-2,6-octadienyl ester; Acetic acid geraniol ester; Bay pine (oyster) oil; Geranyl acetate; Geranyl ethanoate; NSC 2584; trans-1-Acetoxy-3,7-dimethyl-2,6-octadiene; trans-3,7-Dimethyl-2,6-octadien-1-yl acetate; trans-Geranyl acetate; β-Geranyl acetate	

Current regulation: /

Clinical data: /

Additional information:

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

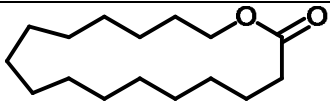
HELIOTROPINE	
CAS # 120-57-0	
EC # 204-409-7	
1,3-Benzodioxole-5-carboxaldehyde	
Piperonal; 2H-Benzo[3,4-d]-1,3-dioxolan-5-ylformaldehyde; 3,4-(Methylenedioxy)benzaldehyde; 3,4-Dihydroxybenzaldehyde methylene ketal; 3,4-Dimethylenedioxybenzaldehyde; 5-Formyl-1,3-benzodioxolane; 5-Formyl-1,3-benzodioxole; 5-Formylbenzodioxole; Benzo[1,3]dioxole-5-carbaldehyde; Benzo[d][1,3]dioxole-5-carboxaldehyde; Heliotropin; Heliotropine; NSC 26826; Piperonaldehyde; Piperonylaldehyde; Protocatechuic aldehyde methylene ether	

Current regulation: /

Clinical data:

In the Frosch 2002 b study, n=2 (0.2%) positive reactions to “piperonal” (1% pet.) and n=6 (0.4%) to “piperonal” (5% pet.), respectively, in 1606 consecutive were observed (17). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% heliotropine in pet., tested in 106 consecutive patients in Barcelona, were observed (15).

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

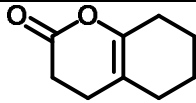
HEXADECANOLACTONE	
CAS # 109-29-5	
EC # 203-662-0	
Oxacycloheptadecan-2-one	
o-Lactone-16-hydroxy-hexadecanoic acid; 1,16-Hexadecanolide; 16-Hexadecanolactone; Cyclohexadecanolide; Dihydroambrettolide; Hexadecanoic acid, 16-Hydroxy-, o-lactone; Hexadecanolactone; Hexadecanolide; Juniperic acid lactone; NSC 33546	

Current regulation: /

Clinical data:

In the Larsen 2001 study, 1 of 178 patients with previously diagnosed contact allergy to fragrance ingredients had a positive PT reaction to this compound, tested 5% pet. (19). In the An 2005 study, 6 of 422 consecutive patients, i.e., 1.4%, had positive reactions to 5% “hexadecanolide” (13).

Additional information: /

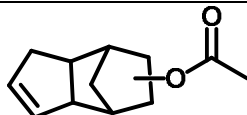
HEXAHYDROCOUMARIN	
CAS # 700-82-3	
EC # 211-851-4	
3,4,5,6,7,8-Hexahydro-2H-1-benzopyran-2-one	
3,4,5,6,7,8-Hexahydro-coumarin; δ -Lactone-2-hydroxy-1-cyclohexene-1-propanoic acid; 3,4,5,6,7,8-Hexahydrocoumarin; Hexahydrocoumarin; Δ -1,6-2-Oxabicyclo(4.4.0)decen-3-one	

Current regulation: Annex II, n° 1135

Clinical data: /

Additional information:

A RIFM review is available (93), p. S115 ff, citing a number of positive human sensitisation experiments.

3α,4,5,6,7,7α-HEXAHYDRO-4,7-METHANO-1H-INDEN-5(OR 6)-YL ACETATE	
CAS # 54830-99-8	
EC # 259-367-2	
3α,4,5,6,7,7α-Hexahydro-4,7-methano-1H-indenol Acetate	
Acetoxidyhydrodicyclopentadiene; Cyclacet; Dicyclopentenyl acetate; Dicylat; Tricyclo[5.2.1.0 ^{2,6}]dec-3-enyl acetate; Tricyclodecenyl acetate	

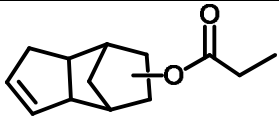
Current regulation: /

Clinical data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 1 to 5% "Cyclacet ®" in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15).

Additional information:

Produced by IFF under the brand name "Cyclacet" (<http://www.iff.com/Ingredients.nsf/0/1C9F2CB39EB1EF6480256993002FBC14>, last accessed 2010-07-08).

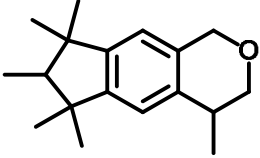
HEXAHYDRO-METHANOINDENYL PROPIONATE	
CAS # 68912-13-0	
EC # 272-805-7	
3α,4,5,6,7,7α-Hexahydro-4,7-methano-1H-indenol propanoate	

3a,4,5,6,7,7a-Hexahydro-4,7-methano-1H-indenyl propionate (Mixture of Isomers); Dicyclopentadiene propionate; tricyclodecenyl propionate; Tricyclo[5.2.1.0 ^{2,6}]dec-3-enyl propionate; Verdyl propionate	

Current regulation: /

Clinical data: /

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

HEXAMETHYLINDANOPYRAN	
CAS # 1222-05-5	
EC # 214-946-9	
1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta[γ]-2-benzopyran	
1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[γ]-2-benzopyrane; 1,3,4,6,7,8-Hexahydro-4,6,6,8,8,8-hexamethylcyclopenta-2-benzopyran; Abbalide; Galaxolide; Galaxolide 50; Galaxolide 50BB; Galaxolide 50IPM; Galaxolide White; HHCB; Pearlide	

Current regulation: /

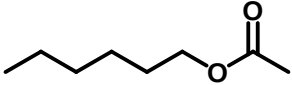
Clinical data:

In the Frosch 2002 a study, n=3 (0.2%) had positive reactions to the compound, tested 10% in isopropyl myristate (with 1 patient reacting positively to the diluent) (16). The Larsen 2001 study, testing with HHCB 7% pet., found 3.4% positive reactions in 178 patients with known contact allergy to fragrance ingredients (19). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had a positive reaction to “Galaxolide 50”, tested at 5% (13) (test concentration 2% pet.). The DeGroot 1985 study identified 3 (1.7%) positive reactions among 179 patients using a 25% PT preparation of HHCB (25). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% “Galaxolide 50 ®” in pet., tested in 100 consecutive patients in Stockholm, were observed (15).

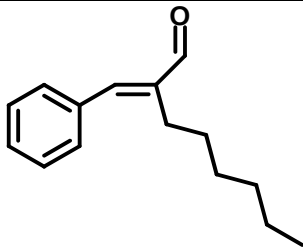
Additional information:

[0403/00 - Opinion concerning Hexahydro-hexamethyl-cyclopenta\(γ\)-2-benzopyran \(HHCB\)](#)

[0610/02 - Opinion on Hexahydro-hexamethyl-Cyclopenta \(γ\)-2-Benzopyran \(HHCB\)](#) (no restrictions) It is a “top 100” substance (IFRA, pers. comm.2010).

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

acid;; NSC 7323; n-Hexyl acetate; n-Hexyl ethanoate	
Current regulation: /	
Clinical data: /	
Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).	

HEXYL CINNAMAL	
CAS # 101-86-0	
EC # 202-983-3	
α-Hexyl-cinnamaldehyde	
2-(Phenylmethylene)octanal; 2-Hexyl-3-phenyl-2-propenal; 2-Hexylcinnamaldehyde; Hexyl cinnamic aldehyde; NSC 406799; NSC 46150; α-Hexylcinnamaldehyde; α-Hexylcinnamic aldehyde; α-Hexylcinnamyl aldehyde; α-n-Hexyl-β-phenylacrolein; α-n-Hexylcinnamaldehyde	
Current regulation: Annex III, part 1, n° 87	

Clinical data:

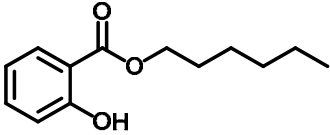
In the “background information” section of the 1999 opinion, hexyl cinnamal (synonymous: alpha-hexyl cinnamal, AHCA) is classified as “less frequently reported allergen”; 2 studies with 1 case and 1 study with 7 cases of contact allergy to this compound in patients with eczema from cosmetic products were found (33).

Since the last SCCNFP-opinion of 1999, in the Frosch 2002 a study, 0.3% positive PT reactions to consecutive patients were noted (16). In the subsequent EU 2005 study, 2 of 1701 patients had positive reactions to AHCA, and n=16 doubtful or irritant to AHCA at 10% in pet. (10). The IVDK 2007 study yielded n=3, i.e. 0.2% (95% CI: 0.03 – 0.4%) positive reactions in 2019 consecutively PTed patients, using 10% pet. as test concentration (4). In the Groningen 2009 study, 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen, using a lower test concentration of 5% pet. (6).

Additional information:

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

Hexyl cinnamal is regarded as “a recommended positive control for skin sensitization testing”, e.g., in the context of the LLNA (118).

HEXYL SALICYLATE	
CAS # 6259-76-3	
EC # 228-408-6	
Hexyl-2-hydroxybenzoate	
Salicylic acid, hexyl ester; 1-Hexyl salicylate; Hexyl salicylate; n-Hexyl salicylate	

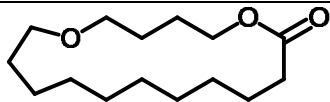
Current regulation: /

Clinical data:

None of the 218 patients with known contact allergy to fragrance ingredients reacted positively to this compound (tested at 5% in pet.) in the Larsen 2002 c study (1).

Additional information:

In a RIFM review, 2 human sensitisation experiments are mentioned which yielded no evidence of sensitising potential (HRIPT, n=103, maximisation test, n=22) (119). It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

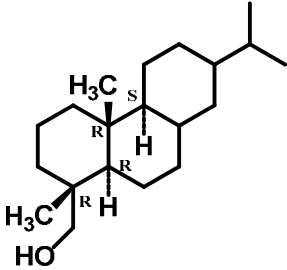
HIBISCOLIDE	
CAS # 6707-60-4	
EC # 229-755-6	
1,6-Dioxacycloheptadecan-7-one	
Undecanoic acid, 11-(4-hydroxybutoxy)-, o-lactone; 12-Oxa-1,16-hexadecanolide; Cervolide; Musk 781; NSC 34741; 12-Oxahexadecan-16-olide	

Current regulation: /

Clinical data:

None of the 178 patients with known contact allergy to fragrance ingredients reacted positively to "12-oxahexadecanolide" (tested at 5% in pet.) in the Larsen 2001 study (19).

Additional information: /

HYDROABIETYL ALCOHOL, when used as a fragrance ingredient	
CAS # 13393-93-6	
EC # 236-476-3	
(1R,4aR,4βS,10aR)-Tetradecahydro-1,4a-dimethyl-7-(1-methylethyl)-1-Phenanthrenemethanol	
Tetradecahydro-1,4a-dimethyl-7-(1-methylethyl)-phenanthrenemethanol; Tetrahydroabietyl alcohol	1-

Current regulation: AnnexII, n° 440

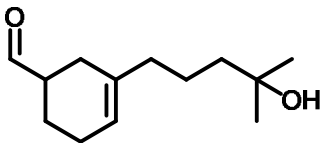
Clinical data:

In the deGroot 2000 study, 17 of 1825 consecutively tested patients had positive reactions to hydroabietyl alcohol (10% pet.) (12).

Additional information:

Commercial hydroabietyl alcohol consists of di- and tetrahydroabietyl alcohol together with non-modified colophony (120)

HYDROXYISOHEXYL	3-CYCLOHEXENE	

CARBOXALDEHYDE (HICC) regioisomers	 51414-25-6
CAS # 31906-04-4 / 51414-25-6	
EC # 250-863-4 / 257-187-9	
4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde (31906-04-4) 3-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde (51414-25-6)	

31906-04-4: 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexenecarboxaldehyde; 4-(4-Methyl-4-hydroxyamyl)cyclohex-3-ene carboxaldehyde; Lyrall

Current regulation: Annex III, part 1, n° 79

Clinical data:

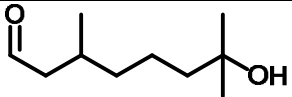
In the “background information” section of the previous opinion (33) HICC is classified as frequent allergen, causing allergic reactions in about 2.8% in consecutive PT patients, two thirds of these being relevant (33).

Since the last SCCNFP-opinion of 1999, in the Frosch 2002 a study, 2.7% of the 1855 consecutive patients reacted positively to HICC (5% pet.) (16). In the EU 2005 study, 28 of 1701 patients (1.7%, 95% CI: 1.1 – 2.4%) reacted positively to 5% HICC in pet. (10). In 21325 patients PTed consecutively in the IVDK 2007 study, 2.4% (95% CI: 2.2 – 2.6%) positive reactions were noted to 5% HICC in pet. (4). Similar to other studies, HICC was the most common single fragrance allergen among 320 patients tested in the Groningen 2009 study, with 3.1% (95% CI: 1.5 – 5.7%) positive reactions despite testing with a lower concentration of 2% pet. (6). In the An 2005 study, 7 of 422 consecutive patients, i.e., 1.7%, had positive reaction (13). The Belsito 2006 study (20) yielded a relatively low prevalence of 0.4% (7 of 1603; exact 95% CI (recalculated): 0.17 – 0.90%) positive reactions with 5% HICC in pet. and even less with lower test concentrations; possible reasons for the much lower prevalence were discussed. The IVDK 2010 study, 2.36% (95% CI: 2.19 - 2.53%) of 37270 consecutively tested patients reacted to HICC (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=8 reacted positively to HICC (48).

Further clinical data with a focus on quantitative dose-response (see also section 4.3), is discussed in (121).

Among the early case reports, S.A. Hendriks reported the case of a 20 year old patient developing axillary dermatitis after 5 months use of a deodorant containing HICC (122).

Additional information: /

HYDROXYCITRONELLAL	
CAS # 107-75-5	
EC # 203-518-7	
7-Hydroxy-3,7-dimethyl-octanal	

(±)-Hydroxycitronellal; 3,7-Dimethyl-7-hydroxyoctanal; 7-Hydroxy-3,7-dimethyloctanal; 7-Hydroxycitronellal; Citronellal hydrate; Citronellal, hydroxy-; Cyclalial; Cyclosia; Cyclosia base; Fixol; Hydroxycitronellal; Laurine; Lilyl aldehyde; Muguet synthetic; Muguetine principle; NSC

406740; Phixia

Current regulation: Annex III, part 1, n° 72

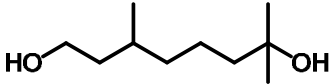
Clinical data:

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	

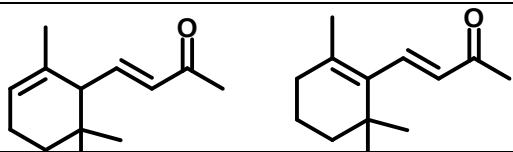
Current regulation: /

Clinical data:

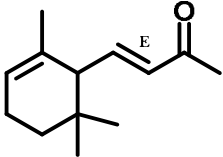
This compound elicited 6.0% positive PT reactions in 218 fragrance sensitive individuals (Larsen 2002 c, (1)).

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.	

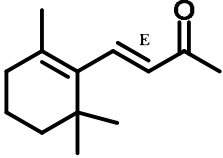
<i>alpha-IONONE</i>	
CAS # 127-41-3	
EC # 204-841-6	
(3E)-4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-Buten-2-one	
(E)-4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-Buten-2-one; (5E)-Ionone; (E)-4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-buten-2-one; (E)-α-Ionone; (±)-trans-α-Ionone; (±)-α-Ionone; 4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-buten-2-one;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

Current regulation: /

Clinical data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% alpha-ionone in pet., tested in 205 consecutive patients, were observed (15).

Additional information: A RIFM review is available (125).

<i>beta-IONONE</i>	
CAS # 79-77-6	
EC # 201-224-3	
(3E)-4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-3-buten-2-one	
(E)-4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-3-buten-2-one; (E)-β-Ionone; Ionone beta; trans-β-Ionone; β-Ionone	

Current regulation: /

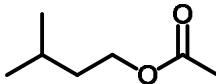
Clinical data:

In the Frosch 1995 dose finding pilot study, no positive reaction to 1% and 5% beta-

ionone in pet., tested in 205 consecutive patients, were observed (15).

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (126).

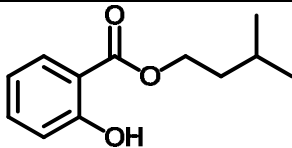
ISOAMYL ACETATE	
CAS # 123-92-2	
EC # 204-662-3	
3-Methylbutyl acetate	
1-Butanol, 3-methyl-, acetate; Acetic acid, isoamyl ester; Isopentyl alcohol, acetate; 3-Methyl-1-butanol acetate; 3-Methyl-1-butyl acetate; 3-Methylbutyl acetate; 3-Methylbutyl ethanoate; Acetic acid 3-methyl-1-butyl ester; Acetic acid 3-methylbutyl ester; Acetic acid isopentyl ester; Banana oil; Isoamyl acetate; Isoamyl alcohol acetate; Isoamyl ethanoate; Isopentyl acetate; Isopentyl ethanoate; NSC 9260; Pear oil; i-Amyl acetate; iso-Amyl acetate; iso-Pentyl acetate	

Current regulation: /

Clinical data: /

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

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

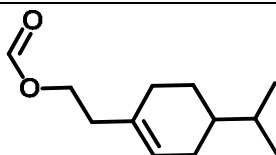
ISOAMYL SALICYLATE	
CAS # 87-20-7	
EC # 201-730-4	
3-Methylbutyl-2hydroxybenzoate	
Isopentyl 2-Hydroxybenzoate; Isopentyl salicylate; Salicylic acid, isopentyl ester (6CI,8CI); Isopentyl alcohol, salicylate; 3-Methylbutyl salicylate; Isoamyl o-hydroxybenzoate; Isoamyl salicylate; Isopentyl salicylate; NSC 7952	

Current regulation: /

Clinical data:

The DeGroot 1985 study identified 1 (0.6%) positive reactions among 179 patients using a 50% PT preparation of this compound – this reaction may have been due to an “excited back syndrome” and is thus a limited evidence (25). In the Frosch 1995 dose finding pilot study, no positive reaction to 1% and 5% isoamyl salicylate in pet., tested in 95 consecutive patients, were observed (15).

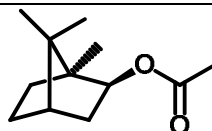
Additional information: A RIFM review is available (127).

ISOBERGAMATE	
CAS # 68683-20-5	
EC # 272-066-0	
4-(Isopropyl)cyclohexadiene-1-ethyl formate	
Structure is incompletely defined 4-(1-Methylethyl)-1,?-cyclohexadiene-1-ethyl formate 4-(Isopropyl)cyclohexadiene-1-ethyl methanoate; menthadienyl formate; Menthadiene-7-methyl formate	

Current regulation: Annex III, part 1, n° 170

Clinical data: /

Additional information: A RIFM review is available (128).

ISOBORNYL ACETATE	
CAS # 125-12-2	
EC # 204-727-6	
(1R,2R,4R)-1,7,7-trimethyl-Bicyclo[2.2.1]hept-2-yl acetate	
Bicyclo[2.2.1]heptan-2-ol, 1,7,7-Trimethyl-, acetate, (1R,2R,4R)-rel- ; Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, exo-; Isoborneol, acetate; (±)-Isobornyl acetate; Isobornyl acetate; NSC 62486; Pichtosin; Pichtosine; exo-Bornyl acetate	

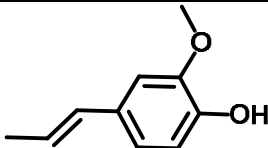
Opinion on fragrance allergens in cosmetic products

Current regulation: /

Clinical data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% isobornyl acetate in pet., tested in 107 consecutive patients in High Wycombe, were observed (15).

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

ISOEUGENOL	
CAS # 97-54-1	
EC # 202-590-7	
2-Methoxy-4-(1-propen-1-yl)-phenol	
Phenol, 2-methoxy-4-(1-propenyl)- ; Phenol, 2-methoxy-4-propenyl-; 1-(3-Methoxy-4-hydroxyphenyl)-1-propene; 2-Methoxy-4-(1-propenyl)phenol; 2-Methoxy-4-propenylphenol; 3-Methoxy-4-hydroxy-1-propenylbenzene; 4-Hydroxy-3-methoxy-1-propenylbenzene; 4-Hydroxy-3-methoxy-β-methylstyrene; 4-Propenylguaiacol; Isoeugenol; NSC 6769	

Current regulation: Annex III, part 1, n° 73

Clinical data:

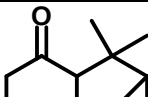
In the “background information” section of the previous opinion (33), isoeugenol, one of the 8 components of the FM I, is classified as frequent allergen, causing allergic reactions in about 1.9% in consecutive PT patients and accounting for 6 to 22% of reactions to the FM I. Allergic reactions had been observed in 2 – 25% of patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 1.3% (95% CI: 0.8 – 1.8%) positive reactions in 2063 consecutively PTed patients (4). In the Groningen 2009 study, 1.3% (95% CI: 0.3 – 3.2%) had positive reactions to isoeugenol, tested at 2% pet., i.e., twice the commonly used concentration (6). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 5.4% positive reactions (22). At St Johns Institute of Dermatology in London 3636 subjects were patch tested with isoeugenol 2001-2005, 97 of whom were positive. Year-on-year incidence showed an increasing trend, with an overall incidence of 2.67% (129). The IVDK 2010 study, 1.62% (95% CI: 0.87 – 2.38%) of 1214 consecutively tested patients reacted to the compound, while 3.41% (95% CI: 2.90 – 3.92%) of 5747 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=11 reacted positively to isoeugenol (48).

Additional information:

Isoeugenol occurs in a cis- (CAS 5912-86-7) and a trans-isomers (CAS 5932-68-3), the latter dominating in trade products (82-88%) (53).

Isoeugenyl methyl ether caused 7.3% positive reactions in the Larsen 2002 c study (1). A number of derivatives of isoeugenol, such as isoeugenyl acetate, transisoeugenol, isoeugenyl benzoate, isoeugenyl phenylacetate, isoeugenyl methyl ether and benzyl isoeugenyl have been examined in 2261 consecutive patients; a varying proportion of positive patch test reactions and a varying proportion of concomitant reactions with isoeugenol have been observed (130). In an earlier study, 5 of 7 patients positive to isoeugenol also displayed positive reactions to isoeugenol acetate (1.2% eth.) (131) (see also section 5 and 6).

ISOLONGIFOLENEKETONE	
CAS # 33407-62-4	

EC # 245-890-3	
1,3,4,6,7,8a-Hexahydro-1,1,5,5-tetramethyl-2H-2,4a-methanonaphthalen-8(5H)-one	
Hexahydro-1,1,5,5-tetramethyl-2H-2,4a-methanonaphthalen-8(5H)-one	

Current regulation: /

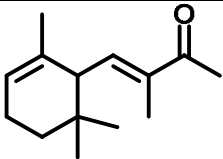
Clinical data:

The Larsen 2001 study identified 1 in 178 patients with known contact allergy to fragrance ingredients who reacted positively in the PT (5% pet.) (19).

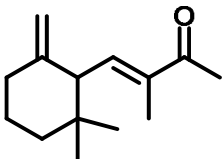
Additional information:

Not listed in CosIng under this CAS #. Other CAS # reported in RIFM review ¹³:

- 29461-14-1 CosIng: INCI name "ISOLONGIFOLENE KETONE EXO";
- 23787-90-8 CosIng: INCI name "ISOLONGIFOLANONE";
- 29461-13-0: CosIng: INCI name "HEXAHYDRO-TETRAMETHYLMETHANONAPHTHALEN-8-ONE".

<i>alpha-ISOMETHYL IONONE</i>	
CAS 127-51-5	
EC 204-846-3	
3-Methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	
4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-methyl-3-buten-2-one; Cetone Alpha; Isomethyl-α-ionone; NSC 66432; α-Cetone	

Current regulation: Annex III, part 1, n° 90

<i>gamma-Methylionone</i>	
CAS 7388-22-9	
EC /	

According to CosIng, "alpha-ISOMETHYL IONONE" (CAS # 127-51-5) and "gamma-Methylionone" (CAS # 7388-22-99) are synonyms, with one CAS number, and one preferred chemical name. The substance(s) are accordingly treated in the 1999 opinion (33) as one. As this treatment is also found in the literature, both substances are reviewed together.

¹³ Opdyke, D. L. J.; Letizia, C. **Monographs on fragrance raw materials. Isolongifolanone.** Food and Chemical Toxicology (1983), 21(6), 859

Clinical data:

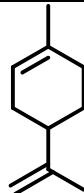
In the “background information” section of the 1999 opinion, “gamma-methylionone” is classified as “less frequently reported allergen”; 1 study with 2 cases and 2 studies with 1 case were found among patients with eczema from cosmetic products (33).

The IVDK 2007 study yielded $n=1$, i.e. 0.1% (95% CI: 0.00 – 0.2%) positive reactions in 2004 consecutively PTed patients (4). In the subsequent period (2005-2008), $n=986$ patients were tested in the IVDK 2010 study, with no positive reactions (7). In the Groningen 2009 study, $n=2$, i.e. 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen, tested at only 1% pet. (6). In a Korean study with 422 consecutive patients, 2.1% reacted positively to “alpha isomethyl ionone (gamma-methylionone), CAS # 127-51-5”, tested 5% pet. (13)

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010) under the label of “alpha-ISOMETHYL IONONE (CAS # 127-51-5)”.

A RIFM review is available, listing 4 human sensitisation experiments employing different study protocols – all yielding negative results (132). Another review is available by Hostynek and Maibach (133), both referring to “alpha-ISOMETHYL IONONE (CAS # 127-51-5)”.

(DL)-LIMONENE	
CAS # 138-86-3	
EC # 231-732-0	
1-Methyl-4-(1-methylethenyl)-cyclohexene	
p-Mentha-1,8-diene; (±)-Dipentene; (±)-Limonene; (±)-α-Limonene; 1,8-p-Menthadiene; 1-Methyl-4-(1-methylethenyl)cyclohexene; 1-Methyl-4-isopropenyl-1-cyclohexene; 1-Methyl-4-isopropenylcyclohexene; 1-Methyl-p-isopropenyl-1-cyclohexene; 4-Isopropenyl-1-methyl-1-cyclohexene; 4-Isopropenyl-1-methylcyclohexene; Cajeputen; Cajeputene; Cinen; Cinene; DL-Limonene; Dipenten; Dipentene; Eulimen; Flavor orange; Goldflush II; Kautschin; Limonen; Limonene; NSC 21446; NSC 844; Nesol; Orange X; Orange flavor; PC 560; Roti-Histol; SF 001; dl-Limonene; α-Limonene	

Current regulation: Annex III, part1, n° 88, 167, 168

Clinical data:

In the “background information” section of the 1999 opinion, d-limonene (CAS 5989-27-5) is classified as “less frequently reported allergen in relation to cosmetic exposure”; with contact allergy to oxidised limonene not infrequently reported in the literature (33).

Since 1999, several studies have been performed using limonene where the oxidation state is not given, but intended to be low. In one study, 0.6% positive reactions to limonene (3% pet.) were observed in 1606 consecutive patients (17). The IVDK 2007 study yielded n=3, i.e. 0.1% (95% CI: 0.03 – 0.4%) positive reactions in 2396 patients consecutively PTed with limonene (2% pet.) (4). The IVDK 2010 study, 0.28% (95% CI: 0 – 0.57%; percentages standardised for age and sex) of 1241 patients PTed with dipentene reacted to the compound (7). In the Groningen 2009 study, no positive reactions to this allergen, tested at 2% pet., were observed in 320 patients (6).

Regarding selected case reports, a case of a 40 year old citrus fruit picker with work related hand dermatitis and bronchial asthma has been described, who tested extreme positive to DL-limonene (2% pet.), and, less extremely, to citronellol and to the biocide dichlorophene (134). Moreover, limonene is used as a solvent in technical applications and cleaning and can lead to allergic contact dermatitis (e.g., a histopathology technicians (135, 136) or a painter and decorator (137)). In “water-free” hand cleansers it is reported to be used in concentrations around 10 – 20% (137). Wax polishes may contain dipentene and have caused one reported case of occupational ACD in a car mechanic (138). Another case of occupational ACD from dipentene in honing oil has been reported (139). In a case series from Sweden, 2 of 105 car mechanics patch tested for occupational contact dermatitis had positive reactions to oxidised d-limonene (5% pet.) (140).

Additional information:

Limonene is a monocyclic monoterpene existing in two enantiomers: (R)-(+)-limonene (CAS 5989-27-5) and (S)-(-)-limonene (CAS 5989-54-8). Racemic limonene is known as dipentene.

The allergenicity of limonene is closely related to oxidation (71, 72, 141, 142). It has been demonstrated that both enantiomers, R-(+)- and S-(-)-limonene spontaneously autoxidize, and that the primary oxidation products formed, the hydroperoxides, are strong and clinically relevant contact allergens. Among 2411 consecutive patients in a multi-centre European study, 63 (2.6 % [95%CI: 2.0-3.3]) reacted to oxidised (R)-(+)-and/or (S)-(-)-limonene (3.0% pet.) (72). In other multi-studies also, a considerable proportion of patients showed

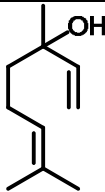
positive patch test reactions to oxidised R-(+)- limonene, e.g.,

- between 0.3% and 5.1% of subgroups of 2800 patients in Stockholm and Leuven, depending on test concentration, oxidation state and department(141),
- between 0.3% and 6.5% in 4 different departments in altogether 2273 patients (72, 143).

The primary oxidation products are the major allergens forming specific antigens (Bråred-Christensson J, Matura M, Bäcktorp C, Börje A, Nilsson JLG, Karlberg A-T. Hydroperoxides form specific antigens in contact allergy. Contact Dermatitis 2006; 55: 230-237.).

Current IFRA standards emphasise “a peroxide value of less than 20 millimoles peroxides per litre, determined according to the FMA method” (<http://www.ifraorg.org/Home/Code,+Standards+Compliance/IFRA+Standards/page.aspx/56>, last accessed 2009-11-11). For a more general discussion see section 5.

There is no scientific rationale for the difference in peroxide value allowed for limonene (20 millimoles peroxides per litre) compared to linalool (10 millimoles peroxides per litre). Specific values for hydroperoxides, which are allergens, would be desirable.

LINALOOL	
CAS # 78-70-6 (isomeric mixture)	
EC # 201-134-4; 245-083-6	
See: http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=27933	
3,7-Dimethyl-1,6-octadien-3-ol	
(±)-Linalool; 2,6-Dimethyl-2,7-octadien-6-ol; 2-Methyl-1-prenyl-3-buten-2-ol; 3,7-Dimethyl-1,6-octadiene-3-ol; 3,7-Dimethyl-3-hydroxy-1,6-octadiene; L 260-2; Linalol; Linalool; Linalyl alcohol; Linanol; NSC 3789; dl-Linalool; β-Linalool	

Current regulation: Annex III, part 1, n° 84

Clinical data:
In the “background information” section of the 1999 opinion, linalool in non-oxidized form is classified as “less frequently reported allergen”; with 4 cases of contact allergy reported in 2 studies on patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, studies have been performed on contact allergy to linalool, oxidation state not given, but intended to be low. In the Larsen 2002 c study, none of the 218 patients with known contact allergy to fragrance ingredients had a positive reaction to linalool 5% pet., as prepared specially for this study (1). The IVDK 2007 study yielded 0.3% (95% CI: 0.1 – 0.6%) positive reactions in 2401 patients consecutively tested with stabilised linalool (10% pet.) (4). The IVDK 2010 study, 1 patient had a weak, and another a ++ reaction among the n=985 patients tested with 10% linalool (stabilised) in pet. (7). In the Groningen 2009 study, n=2, i.e. 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen (6). The deGroot 2000 study with 1825 consecutively tested patients yielded 3 positive reactions to linalool (12). The DeGroot 1985 study found no positive reactions among 179 patients using a 30 % PT preparation of linalool (25).

Additional information:

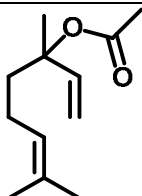
The allergenicity of linalool is closely related to oxidation and the primary oxidation products, the hydroperoxides, are the main allergens (144). In a clinical study 2002-

2003 in 6 European centres including 1511 consecutive patients, 1.3% showed a positive reaction to oxidized linalool (2.0% pet.) and 1.1% to the hydroperoxide fraction (65). A recent dose-response study in Sweden including 3400 patients in two test centres showed a positive reaction in 5.3% of the 1725 patients tested with oxidized linalool 6% pet. (145).

A review by RIFM is available both regarding linalool (146) and linalool “and related esters” (147). Another review is available by Hostynek and Maibach (148).

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

Additional CAS numbers exist for the single isomers: CAS # 126-90-9 (S-isomer), CAS # 126-91-0 (R-isomer); however, in the studies reviewed the isomeric mixture has been used throughout.

LINALYL ACETATE	
CAS # 115-95-7	
EC # 204-116-4	
3,7-Dimethyl-1,6-octadien-3-yl acetat	
1,6-Octadien-3-ol, 3,7-dimethyl-, acetate; Linalool acetate K; (±)-Linaloyl acetate; (±)-Linalyl acetate; 1,5-Dimethyl-1-vinyl-4-hexenyl acetate; 3,7-Dimethyl-1,6-octadien-3-yl acetate; 3-Acetoxy-3,7-dimethyl-1,6-octadiene; Acetic acid linalool ester; Bergamiol; Bergamol; Bergamot mint oil; Linalyl acetate; NSC 2138; dl-Linalool acetate	

Current regulation: /

Clinical data:
In 100 patients tested in Odense, DK, in the early 90s, no positive reactions were observed with 1 and 5% linalyl acetate in pet. (15). In the Frosch 2002 a study, testing with linalyl acetate (10% pet.), 0.2% positive PT reactions to consecutive patients were noted (16). Similarly, the RIFM review mentioned quotes a number of studies where no allergic reactions to this compound had been observed, with the exception of one positive reaction in a Dutch study in 1988(149).

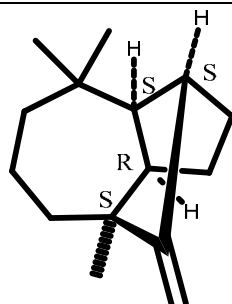
Additional information:

This is the main component of lavender oil (30%), also part of bergamot oil, neroli oil, peppermint oil, lemon oil and jasmine oil (53).

Linalyl acetate autoxidizes spontaneously at air exposure and the major allergens, the hydroperoxides, are the primary oxidation products (150). The pattern of autoxidation is similar to that for linalool and as the acetate can be metabolically hydrolysed to the corresponding alcohol cross reactions to allergens from oxidized linalool should be possible. This was indicated in a study of lavender oil and oxidised linalyl acetate which elicited positive PT reactions in some patients with known contact allergy to oxidised linalool (n=3) (151).

A RIFM review is available reporting 7 human sensitisation experiments yielding few or no cases of sensitisation (152).

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

Longifolene	
CAS # 475-20-7	
EC # 207-491-2	
(1S,3aR,4S,8aS)-Decahydro-4,8,8-trimethyl-9-methylene-1,4-methanoazulene	
1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, (1S,3aR,4S,8aS)-(+)-; 1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, [1S-(1α,3aβ,4α,8aβ)]-; (+)-Longifolene; Junipen; Junipene; Kuromatsuen; Kuromatsuene; Longifolen; NSC 150808; d-Longifolene; α-Longifolene	

Current regulation: /

Clinical data: /

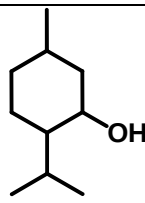
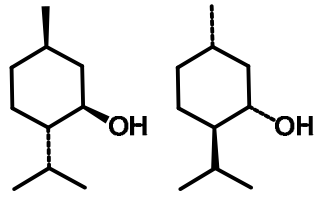
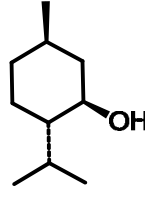
Additional information:

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

http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=77412

This substance is listed in the Register of Flavouring Substances pursuant to Article 3(1) of Regulation EC No. 2232/96 (28 Oct 1996) that lays down a procedure for flavouring substances used or intended for use in or on foodstuffs. Adopted February 23, 1999.

A RIFM review is available citing one negative human maximisation test (n=25) with 10% pet. (153).

MENTHOL	 1490-04-6
CAS # 1490-04-6 / 89-78-1 / 2216-51-5	
EC # 216-074-4 / 239-388-3 / 218-690-9	
5-Methyl-2-(1-methylethyl)-cyclohexanol (1490-04-6) (1R,2S,5R)-rel-5-Methyl-2-(1-methylethyl)-cyclohexanol (89-78-1) (1R,2S,5R)-5-Methyl-2-(1-methylethyl)-cyclohexanol (2216-51-5)	
Other names: 1490-04-6: Menthol; 1-Methyl-4-isopropyl-3-cyclohexanol; 2-Isopropyl-5-methylcyclohexan-1-ol; 2-Isopropyl-5-methylcyclohexanol; 3-Hydroxy-p-menthane; 5-Methyl-2-(1-methylethyl)cyclohexanol; 5-Methyl-2-isopropylcyclohexanol; Menthyl alcohol; p-Menthan-3-ol 89-78-1: (1a,2β,5a)-5-Methyl-2-(1-methylethyl)-cyclohexanol; cis-1,3,trans-1,4-Menthol; dl-Menthol; (1R,2S,5R)-rel-5-Methyl-2-(1-methylethyl)cyclohexanol; (±)-Menthol; DL-Menthol; Fisherman's Friend Lozenges; Hexahydrothymol; Menthacamphor; Menthol; Menthomenthol; NSC 2603; Peppermint camphor; Racementhol; Therapeutic Mineral Ice; Thymomenthol; rac-Menthol	 89-78-1  2216-51-5

2216-51-5: (1R,2S,5R)-5-Methyl-2-(1-methylethyl)-cyclohexanol; [1R-(1 α ,2 β ,5 α)]-5-Methyl-2-(1-methylethyl)-cyclohexanol; (1R,3R,4S)-(-)-Menthol; (-)-Menthol; (-)-Menthyl alcohol; (-)-trans-p-Methan-cis-3-ol; (1R)-(-)-Menthol; (1R,2S,5R)-(-)-Menthol; (1R,2S,5R)-2-Isopropyl-5-methylcyclohexan-1-ol; (1R,2S,5R)-2-Isopropyl-5-methylcyclohexanol; (R)-(-)-Menthol; 1R-Menthol; L-Menthol; L-Mentholum; Levomenthol; NSC 62788; l-(-)-Menthol; l-Menthol

Current regulation: /

Clinical data:

Among 512 patients referred from a dental department for diagnostic work-up of various intraoral symptoms and complaints within 4 years, 10 patients had positive (+ to +++) PT reactions to menthol 5% pet. at D4, mostly reporting dramatic improvement after cessation of use of peppermint-containing oral products (154). In 63 patients positive to the FM I, 1 had a positive PT reaction to menthol, 5% pet., in the Santucci 1987 study (28). The IVDK 2010 study, 1 of 1147 patients tested with 1% menthol in pet. had a weak positive reaction to menthol (7).

A case of contact allergy to “peppermint and menthol” in a transdermal therapeutic system with flurbiprofen for lumbar pain has been described (155). Moreover, a case of rhinitis caused by different menthol-containing products, diagnostically proven by repeatedly positive urticarial reactions after application of 2% menthol in pet. or 5% peppermint oil in pet., has been reported (156). “A case of asthma due to menthol is reported in a 40-year-old woman with no history of asthma or any other allergy. During the last two years, the patient had presented dyspnoea, wheezing and nasal symptoms when exposed to mentholated products such as toothpaste and candies. The aetiology was suggested by the history of exposure and diagnosis was established by skin tests and bronchial challenge with menthol. The patient achieved control of symptoms by avoiding menthol and its derivatives.” (157).

Additional information:

Menthol is an ingredient of several essential oils, like peppermint oil, and has been identified as causative allergen in case reports listed above.

Four stereoisomeric forms are known. Natural menthol occurs as L-form (CAS 2216-51-5), trade products are DL-menthol (CAS 1490-04-6). D-form: CAS 89-78-1, racemic: CAS 15356-70-4. Sensitive to light, air and heat (53).

L-menthol and menthol (isomer not specified) are “top 100” substances (IFRA, pers. comm.2010). RIFM reviews are available regarding “menthol” (158), D-menthol (159), L-menthol (160), DL-menthol (161) and menthol, racemic (162). A CIR expert panel review is available (163).

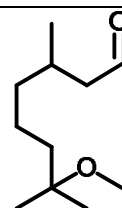
METHOXYCITRONELLAL

CAS # 3613-30-7

EC # 222-784-5

7-Methoxy-3,7-dimethyl-octanal

7-Methoxy-3,7-dimethyloctanal; 7-Methoxy-6,7-dihydrocitronellal; 7-Methoxycitronellal; Methoxycitronellal; Methoxydihydrocitronellal

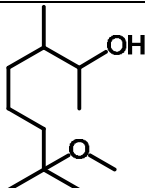


Current regulation: /

Clinical data:

Nakayama et al. found 1974 (after (29)) 12 “strong positive” and 10 “weak positive” reactions to methoxycitronellal (unknown test concentration), with cross-reactions to hydroxycitronellal (proportion not given), in 183 patients.

Additional information: /

METHOXYTRIMETHYLHEPTANOL	
CAS # 41890-92-0	
EC # 255-574-7	
7-Methoxy-3,7-dimethyl-2-octanol	
3,7-Dimethyl-7-methoxy-2-octanol; Dihydromethoxyelgenol; Elesant; Osyrol	

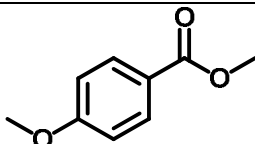
Current regulation: /

Clinical data:

In the Larsen 2002 c study, 0.9% of the patients with known contact allergy to fragrance ingredients had a positive PT reaction to this ingredient not reported as allergen previously (1).

Additional information:

A RIFM review is available (128) citing 1 negative maximisation test (n=27).

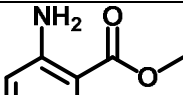
METHYL <i>p</i>-ANISATE	
CAS # 121-98-2	
EC # 204-513-2	
Methyl-4-methoxybenzoate	
p-Anisic acid, methyl ester; 4-(Methoxycarbonyl)anisole; 4-Methoxybenzoic acid methyl ester; Methyl p-anisate; Methyl p-methoxybenzoate; NSC 7324; p-Methoxybenzoic acid methyl ester	

Current regulation: /

Clinical data:

In the Malten 1984 study, n=1 (0.5%) of 182 patients displayed a positive PT reaction to methyl anisate 4% pet. (24).

Additional information: /

METHYL ANTHRANILATE	
CAS # 134-20-3	

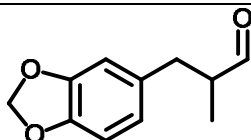
EC # 205-132-4	
Methyl 2-aminobenzoate	
Anthranilic acid, methyl ester; 2-(Methoxycarbonyl)aniline; 2-Aminobenzoic acid methyl ester; 2-Carbomethoxyaniline; Bird Shield; Grain 96-1; Methyl 2-aminobenzoate; Methyl 6-aminobenzoate; Methyl anthranilate; Methyl o-aminobenzoate; NSC 3109; ReJex-iT; Rejex-iT AP 50; Rejex-iT TP 40; Sunarome UVA; [2-(Methoxycarbonyl)phenyl]amine; o-(Methoxycarbonyl)aniline; o-Aminobenzoic acid methyl ester; o-Carbomethoxyaniline	

Current regulation: /

Clinical data:

In 91 Israeli patients with a positive or doubtful reaction to FM I or MP methyl anthranilate was tested (conc. not given), with a negative result (164).

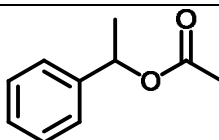
Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

METHYLENEDIOXYPHENYL METHYLPROPANAL	
CAS # 1205-17-0	
EC # 214-881-6	
3-(1,3-Benzodioxol-5-yl)-2-methylpropanal	
Hydrocinnamaldehyde, α-methyl-3,4-(methylenedioxy)-; 2-Methyl-3-(3,4-methylenedioxyphenyl)propanal; 2-Methyl-3-(3,4-methylenedioxyphenyl)propionaldehyde; 3-(3,4-Methylenedioxyphenyl)-2-methylpropanal; Heliobouquet; Heliofresh; 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).

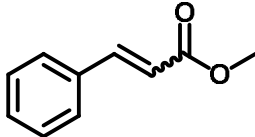
METHYLBENZYL ACETATE	
CAS # 93-92-5	
EC # 202-288-5	
1-Phenylethyl acetate	
Benzenemethanol, α-methyl-, acetate ; Benzyl alcohol, α-methyl-, acetate ; (±)-Styrallyl acetate; (±)-α-Methylbenzyl acetate; (±)-α-Phenethyl acetate; 1-Acetoxy-1-phenylethane; 1-Phenylethyl acetate; Gardeniol II; Gardenol; Methyl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

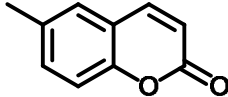
Current regulation: /

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	

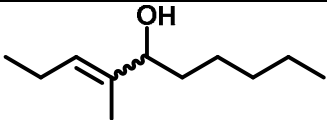
Current regulation: Annex III, part 1, n° 46

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 Portuguese patients tested (168). Similar results (2 of 76 patients with positive photopatchtest) were reported from New York (169).

Additional information: /

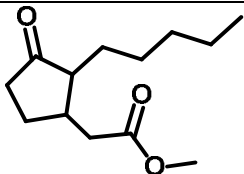
METHYL DECENOL	
CAS # 81782-77-6	
EC # 279-815-0	
4-Methyl-3-decen-5-ol	

Current regulation: /

Clinical data: /

Additional information:

A RIFM review is available (170), reporting 1 negative HRIPT (n=50). It is a “top 100” substance (IFRA, pers. comm.2010).

METHYL DIHYDROJASMONATE	
CAS # 24851-98-7	
EC # 246-495-9	
Methyl 2-(3-oxo-2-pentylcyclopentyl) acetate	

Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester; Kharismal; MDJ; Methyl (3-oxo-2-pentylcyclopentyl)acetate; Methyl 3-oxo-2-pentylcyclopentane ethanoate; Hedione

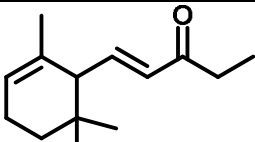
Current regulation: /

Clinical data:

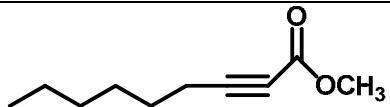
In the Frosch 2002 b study, 3 of 1606 consecutive patients (0.2%) showed positive reactions to hedione (5% pet.) (17). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% hedione in pet., tested in 100 consecutive patients in Belfast, were observed (15).

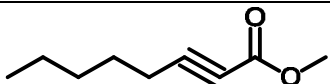
Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). An older RIFM review exists (128) citing 1 negative human maximisation test (n=25).

METHYL IONONE (mixture of isomers)	
CAS # 1335-46-2	
EC # 215-635-0	

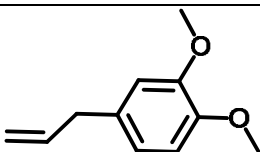
1-(2,6,6-Trimethyl-1-cyclohex-2-enyl)pent-1-en-3-one	
6-Methylionone	
Current regulation: /	
Clinical data:	
See METHYLIONANTHEME for one clinical case report. Regarding methyl ionone gamma, the Frosch 1995 dose-finding pilot study found no positive reaction to 1% and 5% of this substance in pet., tested in 100 consecutive patients in Belfast (15).	
Additional information:	
It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (171).	

METHYL OCTINE CARBONATE	
CAS # 111-80-8	
EC #	
Methyl 2-octynoate	
Methyl 2-Nonynoate, MOC	
Current regulation: Annex III, part 1, n°173	
Clinical data:	
English and Rycroft reported a case of a 19-year-old laboratory technician working in the fragrance industry, who developed hand dermatitis after contact with methyl heptene and methyl octane carbonates; patch testing was strongly positive to both compounds at 1% in MEK (172).	
Additional information: /	

METHYL 2-OCTYNOATE	
CAS # 111-12-6	
EC # 203-836-6	
Methyl oct-2-ynoate	
M2O; Methyl heptin carbonate; Folione; Methyl hept-1-yne-1-carboxylate; Methyl pentylacetylenecarboxylate; NSC 72098; Vert de violette artificiel	
Current regulation: Annex III, part 1, n° 89	

had positive reactions to this allergen, tested at only 2% pet. (6). In a previous case report of a fragrance laboratory assistant with work-related ACD both methyl heptin and methyl octin carbonate had been found sensitisers – probably due to their very similar chemical structure (172). In a recent bi-centric study with 350 eczema patients who were consecutively tested with 1% and 2% M2O in pet.; 0.8% positive reactions were observed. However, in 3 additional cases active sensitisation, with first reactions appearing 2 to 4 weeks after the patch test, and prompt reactions in the 2 cases repeat-patch tested, was observed (174).

Additional information: /

METHYL EUGENOL	
CAS # 93-15-2	
EC # 202-223-0	
1,2-Dimethoxy-4-(prop-2-enyl)benzene	
4-Allylveratrole; Eugenyl methyl ether extra; 1,2-Dimethoxy-4-allylbenzene; 1,3,4-Eugenol methyl ether; 1-(3,4-Dimethoxyphenyl)-2-propene; 1-Allyl-3,4-dimethoxybenzene; 3,4-Dimethoxy-1-(2-propenyl)benzene; 3,4-Dimethoxyallylbenzene; 3-(3,4-Dimethoxyphenyl)propene; 4-Allyl-1,2-dimethoxybenzene; Benzene, 4-allyl-1,2-dimethoxy-; Chavibetol methyl ether; Ent 21040; Eugenol methyl ether; Eugenyl methyl ether; Methyl eugenol ether; Methyl eugenyl ether; Methylchavibetol; NSC 209528; NSC 8900; O-Methyleugenol; Veratrole methyl ether; Veratrole, 4-allyl-	

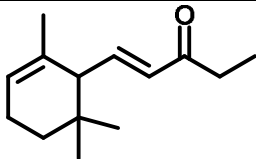
Current regulation: Annex II, 451

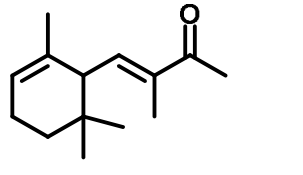
Clinical data:

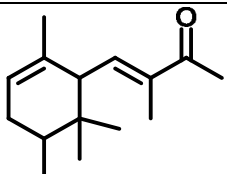
In a previous study by Larsen et al (2002 c), 1.8% of patients with contact allergy to fragrance ingredients reacted positively to this compound (1).

Additional information:

Quote from the SCCS-opinion [0373/00](#): “Methyleugenol should not be intentionally added as a cosmetic ingredient. However, when fragrance compounds containing methyleugenol naturally present in essential oils are used as components in cosmetic products, the highest concentration of methyleugenol in the finished products must not exceed 0.01 % in fine fragrance, 0.004 % in eau de toilette, 0.002 % in a fragrance cream, 0.0002 % in other leave-on products and in oral hygiene products, and 0.001% in rinse-off products.” (The reason is genotoxicity and carcinogenicity).

METHYLIONANTHEME	
CAS # 55599-63-8	
EC #	
(1E)-2-Methyl-1-(2,6,6-trimethyl-2-cyclohexen-1-yl)-1-penten-3-one mixt. with (3E)-3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	

8-Methyl- α -ionone-10-methyl- α -ionone mixt.; Iralia Mixture	
Current regulation: ...	
Clinical	data:
One case of ACD has been reported, caused by an E.d.C. (175).	
Additional information:	
Patented by GIVAUDAN SA 1933, is composed of isomeric n-methylionones and iso-methylionones. Methylionone has CAS # 1335-94-0 (not in CosIng) and 1335-46-2 (METHYL α-IONONE ISOMERS); other names: Methyl-α-cyclocitrilydenacetone; Iralia; Isoaldeine (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.detail&id=41456, last accessed 2010-07-14).	

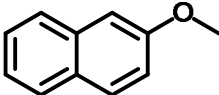
5-METHYL-<i>alpha</i>-IONONE	
CAS # 79-69-6	
EC # 201-219-6	
4-(2,5,6,6-Tetramethyl-2-cyclohexen-1-yl)-3-buten-2-one	
Methyl- α -Ionone; 6-Methyl- α -ionone; α -Irone	
Current regulation: /	

Clinical data:

In the Frosch 2002 b study, 5 of 1606 consecutive patients (0.3%) showed positive reactions to α -irone (10% pet.) (17).

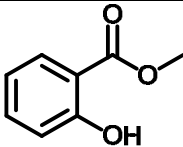
Additional information:

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

METHYL <i>beta</i>-NAPHTHYL ETHER	
CAS # 93-04-9	
EC # 202-213-6	
2-Methoxynaphthalene	
beta-Naphthyl methyl ether; methyl 2-naphthyl ether; Nerolin (old); NSC 4171; Yara yara; β-Methoxynaphthalene; β-Naphthol methyl ether; β-Naphthyl methyl ether; 2-Methoxynaphthalene; Methyl β-naphthyl ether; 2-Naphthol methyl ether; 2-Naphthyl methyl ether; 6-Methoxy-2-naphthalene	
Current regulation: /	

Clinical data: /

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

METHYL SALICYLATE	
CAS # 119-36-8	
EC # 204-317-7	
Methyl 2-hydroxybenzoate	
Other names: Salicylic acid, methyl ester; 2-(Methoxycarbonyl)phenol; 2-Carbomethoxyphenol; 2-Hydroxybenzoic acid methyl ester; Analgit; Anthrapole ND; Ben Gay; Exagien; Flucarmit; Methyl ester of 2-hydroxy benzoic acid; Methyl o-hydroxybenzoate; Methyl salicylate; NSC 8204; Wintergreen oil; o-Hydroxybenzoic acid methyl ester; "Oil of wintergreen"	

Current regulation: /

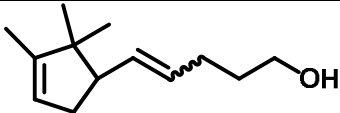
Clinical data:

The deGroot 2000 study yielded 7 positive reactions to methyl salicylate (2% pet.) in 1825 consecutive patients (12).

A case of ACD following the application of a compress bandage containing methyl salicylate has been reported, using 2% "o.o." as PT concentration; the dose per area of methyl salicylate in the occlusive bandage was not reported (177). A similar case was reported in 1977, positive to 2% methyl salicylate in olive oil, with elicitation of pruritus and erythema after oral ingestion of acetyl salicylic acid (178).

Additional information:

A RIFM review is available (179) providing an overview on 3 human sensitisation experiments (e.g., the HRIPT) which were all negative, and clinical data. In a number of older PT studies, positive test results were seen in 6 of 4600, 3 of 183, 3 of 241, 17 of 585, 1 of 70, all employing a test concentration of 2%, usually in pet., according to above review. Methyl salicylate may occur in topical analgesic (OTC) medications, in Germany, for instance, in "Camphopin® Salbe" („Rote Liste 2010").

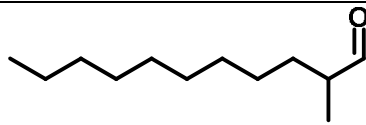
3-METHYL-5-(2,2,3-TRIMETHYL-3-CYCLOPENTENYL)PENT-4-EN-2-OL	
CAS # 67801-20-1	
EC # 267-140-4	
3-Methyl-5-(2,2,3-trimethyl-1-cyclopent-3-enyl)pent-4-en-2-ol	
3-Methyl-5-(2,2,3-trimethyl-3-cyclopenten-1-yl)-4-penten-2-ol; 3-Methyl-5-(2,2,3-trimethylcyclopent-3-enyl)pent-4-en-2-ol; Ebanol	

Current regulation: /

Clinical data:

In the Larsen 2001 study, 1 of 178 patients with known contact allergy to fragrance ingredients exhibited a positive PT reaction to "MTCP", tested 5% pet. (19). In the An 2005 study, 12 of 422 consecutive patients, i.e., 2.8%, had positive reactions to "ebanol", tested at 5% (13).

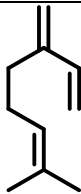
Additional information: /

METHYLUNDECANAL	
CAS # 110-41-8	
EC # 203-765-0	
2-Methylundecanal	
Aldehyde c-12 mna; undecenal, 2-methyl-; 2-Methyl-1-undecanal; Aldehyde M.N.A.; Methyl n-nonyl acetaldehyde; Methylnonylacetaldehyde; NSC 46127	

Current regulation: /

Clinical data: /

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

MYRCENE	
CAS # 123-35-3	
EC # 204-622-5	
7-Methyl-3-methylideneocta-1,6-diene	
2-Methyl-6-methylene-2,7-octadiene; 7-Methyl-3-methylene-1,6-octadiene; NSC 406264; β-Geraniolene; β-Myrcene	

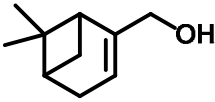
Current regulation: /

Clinical data:

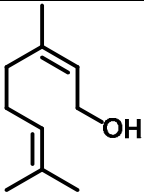
In a clinical study in 6 European centres, including 1511 consecutive patients, 1 patient had a positive reaction to oxidized myrcene (65).

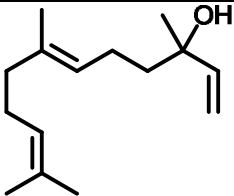
Additional information:

Myrcene autoxidizes spontaneously and rapidly at air exposure. In experimental studies on beta-myrcene an EC3 value of 4.3% was seen for a sample air-exposed 10 weeks (Sköld M. Contact allergy to autoxidized fragrance terpenes (180).

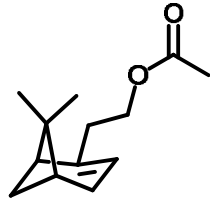
MYRTENOL	
CAS # 515-00-4	
EC # 208-193-5	

(7,7-Dimethyl-4-bicyclo[3.1.1]hept-3-enyl)methanol
(-)-Pin-2-ene-10-ol; 2-Pinen-10-ol; (6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)methanol; (±)-Myrtenol; 6,6-Dimethyl-2-(hydroxymethyl)bicyclo[3.1.1]hept-2-ene; NSC 408846; α-Pinene-10-ol
Current regulation: /
Clinical data: /
Additional information:
A RIFM review exists (181), citing 2 of 3 HRIPT studies with 1 case of sensitisation to myrtenol each.

NEROL	
CAS # 106-25-2	
EC # 203-378-7	
(2Z)-3,7-Dimethylocta-2,6-dien-1-ol	
2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-; (Z)-3,7-Dimethyl-2,6-octadien-1-ol; (Z)-Geraniol; (Z)-Nerol; 2-cis-3,7-Dimethyl-2,6-octadien-1-ol; 3,7-Dimethyl-cis-2,6-octadien-1-ol; Nerol 900; Neryl alcohol; cis-3,7-Dimethyl-2,6-octadien-1-ol; cis-Geraniol; β-Nerol; cis-geraniol – i.e., isomeric to geraniol	
Current regulation: /	
Clinical data:	
In the Larsen 2002 c study, 6.0% of the fragrance sensitive patients reacted positively to 5% in pet. (1).	
Additional information:	
A RIFM review is available (182) citing (negative) human sensitisation experiments, an older study from Japan and the Larsen 2002 c study (see above).	
Regarding autoxidation studies – see geraniol.	

Nerolidol (isomer not specified)	
CAS # 7212-44-4	
EC # 230-597-5	
3,7,11-Trimethyl-1,6,10-odecatrien-3-ol	
Nerolidol; (±)-Nerolidol; FCI 119b; Nerodilol	
Current regulation: /	
Clinical data: /	
Additional information:	

RIFM review is available (183) citing the occurrence of “3 positive reactions in 2273 patients”. Another RIFM review is available on cis-nerolidol (184), mentioning that no data on this compound are available.

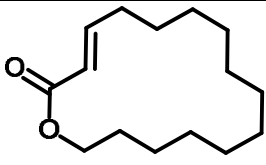
NOPYL ACETATE	
CAS # 128-51-8	
EC # 204-891-9	
2-(7,7-Dimethyl-4-bicyclo[3.1.1]hept-3-enyl)ethyl acetate	
2-Norpinene-2-ethanol, 6,6-Dimethyl-, acetate; Bicyclo[3.1.1]hept-2-ene-2-ethanol, 6,6-dimethyl-, acetate; 2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl acetate; 7,7-Dimethylbicyclo[3.1.1]hept-2-ene-2-ethanol acetate; Citroviol; NSC 1286; NSC 404963; Nopol acetate; Nopyl acetate	

Current regulation: /

Clinical data:

The DeGroot 1985 study identified 2 (1.1%) positive reactions among 179 patients using a 25% PT preparation of this compound – reactions may have at least partly been due to an “excited back syndrome” and thus a limited evidence (25).

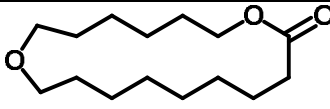
Additional information: /

OXACYCLOHEXADECENONE	
CAS # 34902-57-3	
EC # 609-040-9	
(3E)-Oxacyclohexadec-3-en-2-one	
Globalide; Oxacyclohexadecen-2-one	

Current regulation: /

Clinical data: /

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

OXALIDE	
CAS # 1725-01-5	
EC # 217-033-3	
1,8-Dioxacycloheptadecan-9-one	
Nonanoic acid, 9-[(6-hydroxyhexyl)oxy]-, o-lactone; 10-Oxa-16-hexadecanolide; Oxalide; Oxalide T	

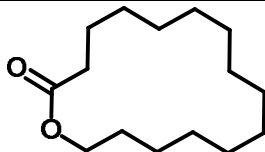
Current regulation: /

Clinical data:

In the Larsen 2001 study, none of 178 patients with known contact allergy to fragrance ingredients exhibited a positive PT reaction to "10-oxahexadecanolide", tested 5% pet. (19).

Additional information:

A RIFM review is available (128), citing a negative maximisation test (n=29).

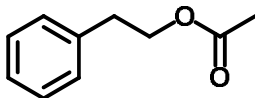
PENTADECALACTONE	
CAS # 106-02-5	
EC # 203-354-6	
1-Oxacyclohexadecan-2-one	
Pentadecanoic acid, 15-hydroxy-, ξ -lactone; 1,15-Pentadecanolide; 15-Hydroxypentadecanoic acid lactone; 15-Pentadecanolide; 15-Pentadodecanolactone; 2-Pentadecalone; CPE 215; Cyclopentadecanolide; Exaltolide; Macrolide Supra; Muskalactone; NSC 36763; Pentadecalactone; Pentadecanolactone; Pentadecanolide; Pentalide; Thibetolide; cpd Supra; ω -Pentadecalactone; angelica lactone; hexaltolide	

Current regulation: /

Clinical data: /

Additional information:

It is a "top 100" substance (IFRA, pers. comm.2010). The substance has been used for clinical olfactory testing in the 60ies under the name of exaltolide.

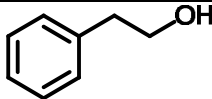
PHENETHYL ACETATE	
CAS # 103-45-7	
EC # 203-113-5	
2-Phenylethyl acetate	
Acetic acid, phenethyl ester ; Phenethyl alcohol, acetate; 2-Phenethyl acetate; 2-Phenylethyl acetate; Benzylcarbiny acetate; NSC 71927; Phenethyl acetate; Phenylethyl ethanoate; β-Phenethyl acetate; β-Phenylethanol acetate; β-Phenylethyl acetate	

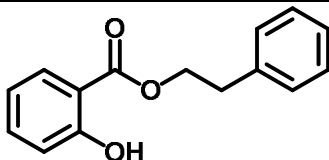
Current regulation: /

Clinical data: /

Additional information:

It is a "top 100" substance (IFRA, pers. comm.2010). Exposure via plants (*Tanacetum parthenium*) is possible (185).

PHENETHYL ALCOHOL	
CAS # 60-12-8	
EC # 200-456-2	
2-Phenylethanol	
Phenethyl alcohol; (2-Hydroxyethyl)benzene; 2-Phenethanol; 2-Phenethyl alcohol; 2-Phenyl-1-ethanol; 2-Phenylethyl alcohol; Benzyl carbinol; Ethanol, 2-phenyl-; NSC 406252; PEA; Phenethanol; Phenethylol; Phenylethanol; Phenylethyl alcohol; β -(Hydroxyethyl)benzene; β -PEA; β -Phenethanol; β -Phenethyl alcohol; β -Phenethylol; β -Phenylethanol; β -Phenylethyl alcohol	
Current regulation: /	
Clinical data:	
The DeGroot 1985 study identified 1 (0.6%) positive reactions among 179 patients using a 25% PT preparation of phenylethyl alcohol (25). In the Frosch 1995 dose-finding pilot study, no positive reaction to this compound, tested 1% pet. in 100 consecutive patients in Odense, DK, was observed (15).	
Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).	

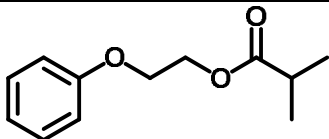
PHENETHYL SALICYLATE	
CAS # 87-22-9	
EC # 201-732-5	
2-Phenylethyl 2-hydroxybenzoate	
Salicylic acid, phenethyl ester; 2-Phenylethyl salicylate; Benzylcarbinyl salicylate; NSC 72035; Phenethyl salicylate	

Current regulation: /

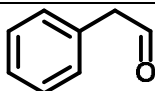
Clinical data: /

Additional information:

A RIFM review exists (186), quoting a negative human maximisation test and a number of animal experiments, including cross-sensitisation experiments with benzyl salicylate. One LLNA study is reported yielding an EC3 value of 2.1%.

PHENOXYETHYL ISOBUTYRATE	
CAS # 103-60-6	
EC # 203-127-1	
2-Phenoxyethyl 2-methylpropanoate	
Isobutyric acid, 2-phenoxyethyl ester; Ethanol, 2-phenoxy-, isobutyrate; 2-Phenoxyethyl isobutyrate; NSC 227210; NSC	

406209; Phenoxyethyl isobutyrate; β -Phenoxyethyl isobutyrate	
Current regulation: /	
Clinical data: /	
Additional information:	
It is a "top 100" substance (IFRA, pers. comm.2010).	

PHENYLACETALDEHYDE	
CAS # 122-78-1	
EC # 204-574-5	
2-Phenylacetaldehyde	
Benzylcarboxaldehyde; Hyacinthin; NSC 406309; Phenacetaldehyde; Phenylacetaldehyde; Phenylacetic aldehyde; Phenylethanal; α-Phenylacetaldehyde; α-Tolualdehyde; α-Toluic aldehyde	

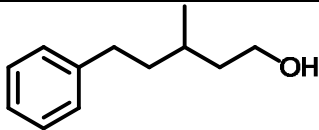
Current regulation: /

Clinical data:

In the Malten 1984 study, 1.1% of 182 patients displayed a positive PT reaction to phenylacetaldehyde 2% pet. (24). In a case report, Sanchez-Politta et al. describe a 26-year-old worker in a perfume factory, who suffered from a spill of pure phenylacetaldehyde and became sensitised, as proven by positive patch tests with 0.5%, 1% and 2% (10 healthy controls negative) (187).

Additional information:

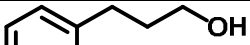
SCCS opinion: [1153/08 - Opinion on "Dermal Sensitization Quantitative Risk Assessment" \(QRA: Citral, farnesol and phenylacetaldehyde\)](#)

PHENYLISOHEXANOL	
CAS # 55066-48-3	
EC # 259-461-3	
3-Methyl-5-phenylpentan-1-ol	
3-Methyl-5-phenyl-1-pentanol; 3-Methyl-5-phenylpentanol; 5-Phenyl-3-methylpentanol; Mefrosol; Phenoxanol	

Current regulation: /

Clinical data: /

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

PHENYLPROPANOL	

Opinion on fragrance allergens in cosmetic products

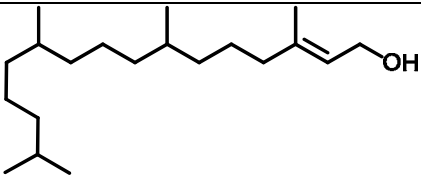
CAS # 122-97-4	
EC # 204-587-6	
3-Phenylpropan-1-ol	
(3-Hydroxypropyl)benzene; 1-Hydroxy-3-phenylpropane; 3-Benzenepropanol; 3-Hydroxy-1-phenylpropane; 3-Phenyl-1-propanol; 3-Phenyl-n-propanol; 3-Phenylpropanol; 3-Phenylpropyl alcohol; Dihydrocinnamyl alcohol; Hydrocinnamic alcohol; Hydrocinnamyl alcohol; NSC 16942; γ-Phenylpropanol; γ-Phenylpropyl alcohol; Phenethyl Carbinol	

Current regulation: /

Clinical data:

The Larsen 2002 c study yielded 0.9% positive reactions in 218 patients with contact allergy to fragrance ingredients (1).

Additional information: ...

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

Current regulation: /

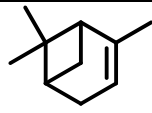
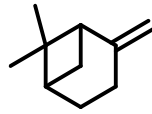
Clinical

data:

/

Additional information:

Phytol is a main constituent of Jasmin abs. with 7.4% reported content (17). In a human maximization study involving 25 subjects, there was one case of contact sensitization to 10% phytol (6900 µg/cm²), applied in petrolatum, as reported in a RIFM review (188).

alpha-PINENE and beta-PINENE	
CAS # 80-56-8 (alpha-Pinene); CAS # 127-91-3 (beta-Pinene)	 80-56-8
EC # 201-291-9 (alpha-Pinene; according to CAS service: 219-445-9); EC # 204-872-5 (beta-Pinene; according to CAS service: 245-424-9)	 127-91-3
2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene (80-56-8)	
6,6-Dimethyl- 2-methylenebicyclo[3.1.1]heptane (127-91-3)	

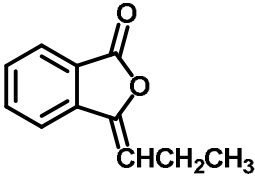
80-56-8: 2-Pinene; (±)-2-Pinene; (±)-α-Pinene; Acintene A; NSC 7727; PC 500; PC 500 (terpene); Sylvapine A; α-Pinene 127-91-3: 2(10)-Pinene ; (±)-2(10)-Pinene; (±)-6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane; (±)-β-Pinene; 6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane; NSC 21447; NSC 406265; NSC 59190; Nopinen; Nopinene; PC 600; PC 600 (pesticide); Pseudopinen; Pseudopinene; Terebenthene; β-Pinene	
Current regulation: Annex III, part 1, n° 130 (Peroxide value less than 10 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 et al. found 14 to be sensitised to "Indonesian oil of turpentine" and 8 to alpha-pinene (190).

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

Additional information: /

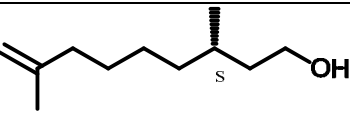
PROPYLIDENE PHTHALIDE	
CAS # 17369-59-4	
EC # 241-402-8	
3-Propylidene-2-benzofuran-1-one	
3-Propylidene-1(3H)-isobenzofuranone; Propylidenephthalide; Celeriax; Propylidenephthalide	

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

Clinical data:

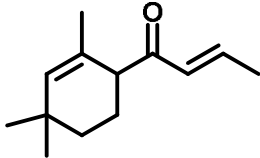
In the Malten 1984 study, 2.6% of 182 patients displayed a positive PT reaction to ethyl acrylate 1% pet. (24). In this paper, "3/25" positive results in human maximisation tests are listed.

Additional information: /

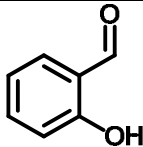
RHODINOL	
CAS # 6812-78-8	
EC # 229-887-4	
(3S)-3,7-Dimethyloct-7-en-1-ol	
Rhodinol; (-)-Rhodinol; α-citronellol; (-)-α-Citronellol; (S)-	

Opinion on fragrance allergens in cosmetic products

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

<i>trans</i>-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.

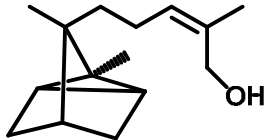
SALICYLALDEHYDE	
CAS # 90-02-8	
EC # 201-961-0	
2-Hydroxybenzaldehyde	
Salicylaldehyde; 2-Formylphenol; NSC 112278; NSC 49178; NSC 83559; NSC 83560; NSC 83561; NSC 83562; NSC 97202; Salicylal; Salicylic aldehyde; o-Formylphenol; o-Hydroxybenzaldehyde	
Current regulation: /	

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).

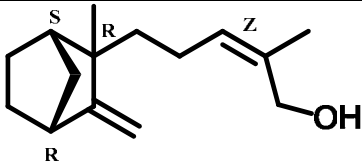
Additional information: Along with other derivates of salicylic acid, salicylaldehyde is found in the bark of several trees, such as willow or aspen, and can cause allergic contact dermatitis by this exposure (196).

<i>alpha-SANTALOL</i>	
CAS # 115-71-9	
EC # 204-102-8	
(R Z)- 5-(2,3-dimethyltricyclo[2.2.1.0^{2,6}]hept-3-yl)-2-methylPent-2-en-1-ol	
2-Penten-1-ol, 5-(2,3-dimethyltricyclo[2.2.1.0 ^{2,6}]hept-3-yl)-2-methyl-, [R(Z)]-; 2-Penten-1-ol, 5-(2,3-dimethyltricyclo[2.2.1.0 ^{2,6}]hept-3-yl)-2-methyl-, stereoisomer; α-Santalol; Tricyclo[2.2.1.0 ^{2,6}]heptane, 2-penten-1-ol deriv.; (+)-(Z)-α-Santalol; (+)-α-Santalol; (Z)-α-Santalol; Sandal; Santalol a; cis-α-Santalol; d-α-Santalol	

Current regulation: /

Clinical data: / (see beta-santalol)

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

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

Current regulation: /

Clinical

data:

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

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

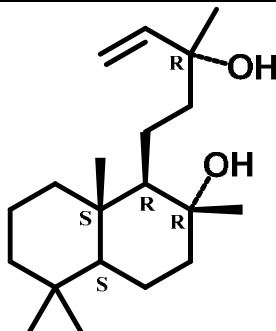
Additional information: “There is no one CAS number for the mixture. The alpha form has a CAS No. 115-71-9 and the beta form is 37172-32-0 (this # is trans-.beta.-santalol). There was no reported use of these materials in the last two IFRA Surveys (8 years total)” (A.M. Api, pers. comm., 2010).

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

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

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

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

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

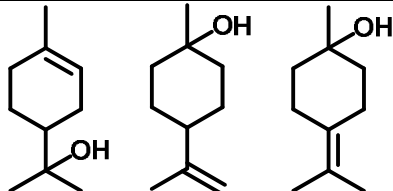
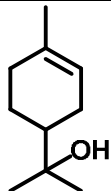
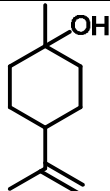
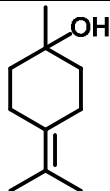
Current regulation: /

Clinical data: /

Additional information:

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

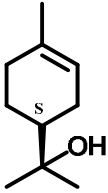
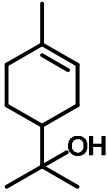
[0986/06 - Opinion on Sclareol \(sensitisation only\)](http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_056.pdf)
(http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_056.pdf)

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

Current regulation: /

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

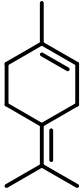
Additional information: It is a "top 100" substance (IFRA, pers. comm. 2010).

<i>alpha-TERPINEOL</i>	 10482-56-1
CAS # 10482-56-1 / 98-55-5	
EC # 233-986-8 / 202-680-6	
2-[(1S)-4-Methyl-1-cyclohex-3-enyl]propan-2-ol (10482-56-1)	
2-(4-Methyl-1-cyclohex-3-enyl)propan-2-ol (98-55-5)	
10482-56-1: (S)-(-)-p-Menth-1-en-8-ol; (-)- α -Terpineol; (S)-(-)-Terpineol; (S)-(-)- α -Terpineol; (S)- α -Terpineol; l- α -Terpineol 98-55-5: p-Menth-1-en-8-ol; ($\pm$)- 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; $\alpha,\alpha,4$ -Trimethyl-3-cyclohexene-1-methanol; α -Terpineol	 98-55-5
Current regulation: /	

Clinical data:

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

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

<i>Terpinolene</i>	
CAS # 586-62-9	
EC # 209-578-0	
1-Methyl-4-propan-2-ylidenecyclohexene	
p-Mentha-1,4(8)-diene; 1-Methyl-4-(1-methylethylidene)-cyclohexene; 4-Isopropylidene-1-methylcyclohexene; Isoterpinene; Nofmer TP; Terpinolen; Terpinolene; α -Terpinolene; δ -Terpinene	

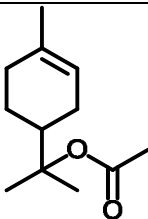
Current regulation: Annex III, part 1, n° 133 (Peroxide value less than 10 mmoles/L in substance)

Clinical data:

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

gave a positive result (negative in 10 controls) (203). Eleven patients sensitised to tea tree oil showed positive reactions to alpha-terpinene, terpinolene and ascaridol (204).

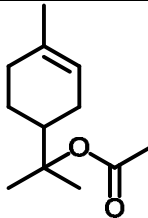
Additional information: It is a “top 100” substance (IFRA, pers. comm. 2010)

TERPINEOL ACETATE (Isomer mixture)	
CAS # 8007-35-0	
EC # 232-357-5	
4-Methyl-1-propan-2-yl-1-cyclohex-2-enyl acetate	
Terpinyl acetate	

Current regulation: /

Clinical data:
In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% terpinyl acetate in pet., tested in 106 consecutive patients in Barcelona, were observed (15)

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

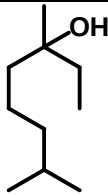
<i>alpha</i>-TERPINYL ACETATE	
CAS # 80-26-2	
EC # 201-265-7	
2-(4-Methyl-1-cyclohex-3-enyl)propan-2-yl acetate	
3-Cyclohexene-1-methanol, α,α,4-trimethyl-, acetate; p-Menth-1-en-8-ol, acetate; (±)-α-Terpineol acetate; (±)-α-Terpinyl acetate; 2-(4-Methyl-3-cyclohexen-1-yl)-2-propyl acetate; Terpinyl acetate; α-Terpineol acetate; p-Menth-1-en-8-yl acetate; 1-Methyl-1-(4-methylcyclohex-3-enyl)ethyl ethanoate; (±)-.alpha.,.alpha.,4-trimethylcyclohex-3-ene-1-methyl acetate	

Current regulation: /

Clinical data:

The DeGroot 1985 study identified no positive reactions among 179 patients using a 10% PT preparation of “terpinyl acetate” (25).

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

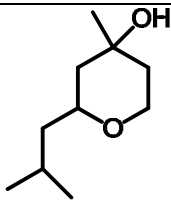
Tetrahydrolinalool	
CAS # 78-69-3	
EC # 201-133-9	
3,7-Dimethyloctan-3-ol	
2,6-Dimethyl-6-octanol; 3,7-Dimethyloctan-3-ol; Linalool tetrahydride; NSC 128151; Tetrahydrolinalool	

Current regulation: /

Clinical data:
/

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

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

TETRAHYDRO-METHYL-METHYLPROPYL)-PYRAN-4-OL	
CAS # 63500-71-0	
EC # 405-040-6	
4- Methyl-2-(2-methylpropyl)tetrahydro-2H-4-pyranol	
2-(2-Methylpropyl)-4-hydroxy-4-methyltetrahydropyran; 2-Isobutyl-4-hydroxy-4-methyltetrahydropyran; 2-Isobutyl-4-methyltetrahydropyran-4-ol; 4-Hydroxy-4-methyl-2-(2-methylpropyl)tetrahydropyran; Florosa; Rozanol	

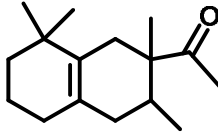
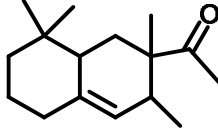
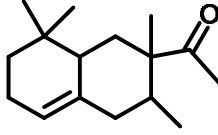
Current regulation: /

Clinical

data:

/

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

TETRAMETHYL ACETYLOCTAHYDRONAPHTHALENES	 54464-57-2
CAS # 54464-57-2 / 54464-59-4 / 68155-66-8 / 68155-67-9	
EC # 259-174-3 / 259-175-9 / 268-978-3 / 268-979-9	
1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)-ethanone (54464-57-2)	
1-(1,2,3,4,5,6,7,8-Octahydro-2,3,5,5-tetramethyl-2-naphthalenyl)-ethanone (54464-59-4)	
1-(1,2,3,5,6,7,8,8a-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)-ethanone (68155-66-8)	 68155-66-8
1-(1,2,3,4,6,7,8,8a-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)-ethanone (68155-67-9)	
54464-57-2: 1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)ethanone; 1',2',3',4',5',6',7',8'-Octahydro-2',3',8',8'-tetramethyl-2'-acetoneaphthone; 7-Acetyl-1,2,3,4,5,6,7,8-octahydro-1,1,6,7-tetramethylnaphthalene; Amberonne; Ambralux; Iso Ambois Super; Iso-E Super; Isocyclemon E; OTNE; Orbitone	
	 68155-67-9

Current regulation: /

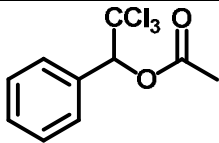
Clinical

data:

In the Frosch 2002 a study, 0.2% of 1855 consecutive patients reacted to the compound (brand name mentioned: „Iso E. Super“, 5% pet.) (16). In the Frosch 1995 dose-finding pilot study, 1 positive reaction both to 1% and 5% “Iso E Super ®” in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15). The Larsen 2001 study yielded 1.7% positive reactions (5% pet.) in 178 patients with known contact allergy to fragrance ingredients (19).

Additional information: According to CosIng: "Mixture of isomers: 1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8-tetramethyl-2-naphthyl)ethan-1-one; 1-(1,2,3,4,5,6,7,8-Octahydro-2,3,5,5-tetramethyl-2-naphthyl)ethan-1-one; 1-(1,2,3,5,6,7,8,8a-Octahydro-2,3,8,8-tetramethyl-2-naphthyl)ethan-1-one (68155-67-9); 1-(1,2,3,4,6,7,8,8a-Octahydro-2,3,8,8-tetramethyl-2-naphthyl)ethan-1-one (68155-66-8) ", (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40504>, last accessed 2009-11-11).

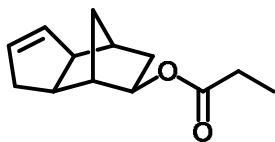
It is a "top 100" substance (IFRA, pers. comm. 2010)

TRICHLOROMETHYL PHENYL CARBINYL ACETATE	
CAS # 90-17-5	
EC # 201-972-0	
2,2,2-Trichloro-1-phenylethyl acetate	
Benzenemethanol, α-(trichloromethyl)-, acetate; Benzyl alcohol, α-(trichloromethyl)-, acetate (Trichloromethyl)phenylcarbinyl acetate; (±)-α-(Trichloromethyl)benzyl acetate; 2-Acetoxy-1,1,1-trichloro-2-phenylethane; Crystal rose; NSC 165582; Rosacetol; Rosephenone; Rosetone; Rosone; α-(Trichloromethyl)benzyl acetate	

Current regulation: /

Clinical data: /

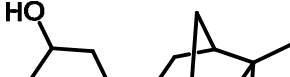
Additional information: It is a "top 100" substance (IFRA, pers. comm. 2010)

TRICYCLODECENYL PROPIONATE	
CAS # 17511-60-3	
EC # 241-514-7	
3α,4,5,6,7,7α-Hexahydro-4,7-methano-1H-inden-6-yl propionate	
4,7-Methano-1H-inden-6-ol, 3α,4,5,6,7,7α-Hexahydro-, propanoate; 4,7-Methanoinden-6-ol, 3α,4,5,6,7,7α-Hexahydro-, propionate; Cyclaprop; Florocyclene; Greenyl propionate; Tricyclo(5.2.1.02,6)dec-3-en-8-yl propionate.	

Current regulation: /

Clinical data: /

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

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

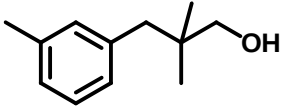
Opinion on fragrance allergens in cosmetic products

CAS # 3407-42-9	
EC # 222-294-1	
3-(5,5,6-Trimethyl-6-bicyclo[2.2.1]heptanyl)cyclohexan-1-ol	
3-(5,5,6-Trimethyl-2-norbornyl)-cyclohexanol; 3-(5,5,6-Trimethylbicyclo[2.2.1]hept-2-yl)cyclohexan-1-ol; 3-Hydroxy-1-(5-isocamphyl)cyclohexane; Sandela	

Current regulation: /

Clinical data: /

Additional information: part of “synthetic sandalwood oil”.

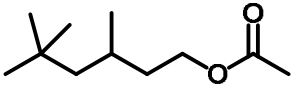
TRIMETHYL-BENZENEPROPANOL (Majantol)	
CAS # 103694-68-4	
EC # 403-140-4	
2,2-Dimethyl-3-(3-methylphenyl)propan-1-ol	
2,2-Dimethyl-3-(3-tolyl)propan-1-ol; 3-(2,2-Dimethyl-3-hydroxypropyl)toluene	

Current regulation: /

Clinical data:

In the Larsen 2002 c study, majantol (conc. not given, elsewhere reported as 5% pet.) caused positive PT reactions in 3.2% of patients with known contact allergy to fragrance ingredients. In a later study by the IVDK, 0.5% (95% CI: 0.3 – 0.7%) consecutive patients displayed a positive reaction to majantol 5% pet. (206). In the IVDK 2010 study, majantol was tested both in n=2189 consecutive patients, yielding 0.36 % (95% CI: 0.12—0.60%) positive reactions, and in the context in a special series, applied in an aimed fashion to n=4972 patients, yielding 0.76% (95% CI: 0.49—1.03%) (standardised) positive reactions (7). In a recent study from Copenhagen, DK, 6 of 722 patients tested with this compound were found positive, 2 of these to material used earlier provided by Symrise, 4 to material by Allmiral/Hermal/Trolab used later instead. There was no significant difference between these proportions obtained with batches of majantol from different production processes (207).

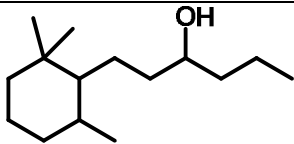
Additional information: /

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

Current regulation: /

Clinical data: /

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

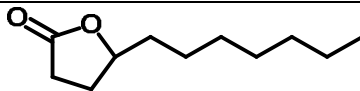
TRIMETHYL-PROPYLCYCLOHEXANEPROPANOL (TMCH)	
CAS # 70788-30-6	
EC # 274-892-7	
1-(2,2,6-Trimethylcyclohexyl)hexan-3-ol	
Other names: 2,2,6-Trimethyl-alpha-propylcyclohexanepropanol (REACH, EINECS); .alpha.-Propyl-2,2,6-trimethyl- cyclohexanepropanol; 6-(2,2,6-Trimethylcyclohexyl)-4- hexanol; Finotimber;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

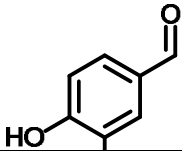
<i>gamma</i>-UNDECALACTONE	
CAS # 104-67-6	
EC # 203-225-4	
5-Heptyltetrahydrofuran-2-one	
Undecanoic acid, 4-hydroxy-, γ -lactone; (RS)- γ -Undecalactone; ($\pm$)- γ -Undecalactone; 4-Hydroxyundecanoic acid lactone; 4-Undecanolide; 5-Heptyldihydro-2(3H)-furanone; NSC 406421; NSC 46118; NSC 76413; Neutralizing agent 350120-1; Peach lactone; Peche Pure; Persicol; γ -(n-Heptyl)- γ -butyrolactone; γ -Heptyl- γ -butyrolactone; γ -Heptylbutyrolactone; γ -Undecalactone; γ -Undecanolactone; γ -Undecanolide; γ -n-Heptylbutyrolactone	

Current regulation: /

Clinical data: /

Additional information:

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

VANILLIN	
CAS # 121-33-5	
EC # 204-465-2	

4-Hydroxy-3-methoxybenzaldehyde	
2-Methoxy-4-formylphenol; 3-Methoxy-4-hydroxybenzaldehyde; 4-Formyl-2-methoxyphenol; 4-Hydroxy-5-methoxybenzaldehyde; 4-Hydroxy-m-anisaldehyde; H 0264; Lioxin; NSC 15351; NSC 403658; NSC 48383; Rhovanil; Vanillaldehyde; Vanillic aldehyde; Vanillum; m-Methoxy-p-hydroxybenzaldehyde; p-Hydroxy-m-methoxybenzaldehyde; p-Vanillin	

Current regulation: /

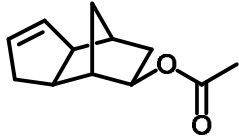
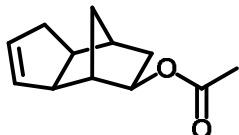
Clinical data:

In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 1 positive reaction to vanillin was observed (3). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=1 (0.1%) positive reaction to vanillin 10 % pet. (22). The IVDK 2010 study, n=10, i.e., 0.19% (95% CI: 0.07 – 0.32%; percentages standardised for age and sex) of 4377 patients PTed reacted to the compound, tested 10% pet. (7). In n=102 patients with a positive reaction to MPR, 19 compounds of this natural mixture were tested, among these, vanillin, to which none reacted positively (208). In 21 patients with contact allergy to propolis, 2 also reacted to vanillin (10% pet.) (209).

A 13-year-old girl with recurrent (peri-)cheilitis after application of a vanilla lip salve tested strongly positive to this salve (as is), "Vanilla 10% pet." (unclear, whether natural extract or vanillin) and MPR (210). Trattner/David identified 1 / 641 consecutive patients with positive reaction to vanillin (31).

Additional information:

Naturally occurring in the fruit of *Vanilla planifolia* after a fermentation process, in styrax, clove oil, potatoes, wood, including Myroxylon pereirae resin, and other material (53). Nowadays, vanillin is synthesised from eugenol, guajakol and lignin residues from paper production, however, not fully achieving the subtle scent and taste of the natural material (53). It is a "top 100" substance and classified as R43 (IFRA, pers. comm. 2010).

VERDYL ACETATE	
CAS # 2500-83-6/ 5413-60-5	
EC # 219-700-4 / 226-501-6	
3a,4,5,6,7,7a-Hexahydro-4,7-methanoinden-6-yl acetat (2500-83-6)	2500-83-6
3a,4,5,6,7,7a-Hexahydro-4,7-methano-1H-inden-5-yl acetat (5413-60-5)	
2500-83-6: 4,7-Methano-1H-inden-5-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; 4,7-Methanoinden-5-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; NSC 142428; NSC 94573	
5413-60-5: 4,7-Methano-1H-inden-6-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; 4,7-Methanoinden-6-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; 4,7-Methano-3a,4,5,6,7,7a-hexahydroinden-6-yl acetate; 8-Acetoxytricyclo[5.2.1.0 ^{2,6}]dec-3-ene; Greenyl acetate;	5413-60-5

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Herbaflorat; Jasmacyclene; NSC 6598	

Current regulation: /

Clinical data: /

Additional information:

In CosIng, both above CAS numbers are listed under “verdyl acetate” (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41289>, last accessed 2010-07-19).

In the CAS, there are 2 separate entries; moreover, there are 2 separate RIFM reviews:

- # 2500-83-6: Other names: Tricyclo[5.2.1.0^{2,6}]dec-4-en-8-yl acetate (REACH, EINECS, INCI Name according to CAS); 3a,4,5,6,7,7a-Hexahydro-4,7-methanoinden-6-yl Acetate; Tricyclodecen-4-yl 8-Acetate. It is a “top 100” substance (IFRA, pers. comm. 2010). A RIFM review is available, stating that “no data is available” regarding the skin sensitising properties of the substance (211).
- # 5413-60-5: Other names: 3a,4,5,6,7,7a-hexahydro-4,7-methanoinden-6-yl acetate (REACH, EINECS, INCI Name according to CAS), 4,7-Methano-3a,4,5,6,7,7a-hexahydroinden-6-yl acetate; 4,7-Methanoinden-6-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; 8-Acetoxytricyclo[5.2.1.0^{2,6}]dec-3-ene; Tricyclodecenyl acetate; Greenyl acetate; Herbaflorat; Jasmacyclene; NSC 6598; Verdyl acetate. It is a “top 100” substance (IFRA, pers. comm. 2010). A RIFM review is available (212), citing 2 negative human maximisation tests and 1 negative HRIPT.

Natural extracts / essential oils

Natural raw materials in terms of extracts are used in the fragrance and flavour industry for various reasons. Most importantly, several naturally occurring mixtures have a very complex composition and sensory nature which cannot (fully) be achieved by synthetic material. Moreover, several compounds cannot be synthesised at a competitive price, and the demand for perfumes based on natural materials is considerable (34).

The three main methods used to concentrate plant fragrance substances as essential oils comprise steam distillation, mechanical processes from the epicarp of Citrus fruits ("pressing") and dry distillation. An Essential oil is „obtained by steam distillation with addition of water in the still (hydrodistillation) or without addition of water in the still (directly by steam“)(213). Essential oil of fruit juice is „obtained by from a fruit juice during its concentration or during UHT (flash pasteurization) treatment“ (213). Cold pressed essential oil is „obtained by mechanical processes from the epicarp of the fruit of a Citrus, at ambient temperature“(213). Citrus peel oils, apart from distilled Citrus oils, are produced with various methods (214). The oil consists of a high volume of volatile terpenes, mostly monoterpenes but also contains small amounts of non-volatile compounds such as dyes, waxes and furocoumarines.

The method of solvent extraction is generally applied in the separation of heat-labile materials or if an essential oil can only be obtained in very low yield, e.g. from blossoms. It is also used if the non-volatile components are desired for their fixative properties, e.g. in the preparation of resinoids from exudates. The most important extracts are termed: (i) concrete: an extract „obtained from a fresh plant natural raw material by extraction with a solvent“¹⁸, containing not only volatile, but also a large proportion of non-volatile substances such as waxes; and (ii) absolute: „product, obtained by extraction with ethanol from a concrete, a floral pomade, a resinoid or a supercritical fluid extract. The ethanolic solution is generally cooled down and filtered in order to eliminate the «waxes»; the ethanol is then eliminated by distillation“¹⁹. Resinoids, used for their fixative properties, are „obtained from a dry plant natural raw material by extraction with a solvent“²⁰. The products are usually highly viscous and thus might sometimes be diluted, e.g. with phthalates or benzyl benzoate. Oleoresins are extracts „of spice or aromatic herb“ by „treating a natural raw material with a solvent, then, after filtration if necessary, the solvent is eliminated“²¹.

Regarding clinical data in terms of contact allergy to fragrance ingredients, the main focus of case report or clinical studies regarding essential oils and natural extracts, respectively, is on general dermatological patients with complaints related to use of cosmetics etc. However, series of cases with occupational exposure to essential oils with occupational allergic contact dermatitis have also been reported (e.g., masseurs,

¹⁸ ISO/DIS 9235

¹⁹ ISO/DIS 9235

²⁰ ISO/DIS 9235

²¹ ISO/DIS 9235

physiotherapists (215, 216), aromatherapists (217-221), beauticians doing massages (222); for further details, e.g., PT results with various essential oils, see original case reports. "Current Regulation" refers to the EU Cosmetics Directive only.

Catalogue of natural extracts / essential oils evaluated

ACORUS CALAMUS ROOT OIL

CAS 84775-39-3; EC 283-869-0

Calamus Oil; "Sweet Flag Oil"

(*Acorus calamus*, ext. = INCI name)

Current regulation: /

Clinical data:

The Rudzki 1976 study found no positive reaction in 200 patients to "calamus" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=7 (8.1%) positive reactions to "calamus" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Acorus calamus* L. (sweet flag calamus). *Acorus Calamus* Root Oil is an essential oil obtained from the rhizomes of the calamus, *Acorus calamus* L., Araceae. It contains beta-asarone (up to 96%, depending on ploidy, and with this, origin (34)), calamene (about 4%), calamol (about 3%) alpha-asarone (about 1%), camphene (about 1%) and some beta-pinene and asaronaldehyde (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41330>, last accessed 2010-01-29. Use is restricted due to potential toxicity of beta-asarone (34).

CANANGA ODORATA and Ylang-ylang oil

Ylang-ylang and cananga oils are essential oils that are obtained from two subspecies of the cananga tree (34). In the INCI nomenclature, both are not differentiated.

CANANGA ODORATA FLOWER EXTRACT

CAS 83863-30-3; EC 281-092-1
(ylang-ylang, ext.) INCI name:
CANANGA ODORATA EXTRACT

CANANGA ODORATA FLOWER OIL

CAS 8006-81-3, 68606-83-7; EC / (oils,
ylang-ylang) INCI name: CANANGA
ODORATA OIL

Current regulation: ...

Clinical data:

Ylang-ylang oil

ISO 4720:2009 nomenclature: *Cananga odorata* (Lam.) Hook. f. et Thomson *forma*

genuina)

In the Larsen 2002 c study, “synthetic ylang-ylang oil” caused 6.4% positive reactions in 218 patients with known contact allergy to fragrance ingredients (1). In a Japanese study, M. Sugawara et al. noted a significant decline of the proportion of patients reacting positively to “ylang-ylang oil 5% pet.” from 1971 to 1989, the overall number in patients with cosmetic dermatitis amounting to 176 of 1438 (12.2%, 95% CI: 10.6 – 14.0%) (223). In the Frosch 2002 b study, two fractions of Ylang-Ylang oil (I and II) were separately tested, each at 10% pet. Fraction I yielded 2.6%, fraction II 2.5% positive test reactions (no data on concomitant reactivity given) (17). The deGroot 2000 study, with 1825 consecutively tested patients, found 18 positive PT reactions to “ylang-ylang oil”, tested at 4% in pet. (12). The Sugiura 2000 study with 1483 patients with suspected cosmetic dermatitis observed 0.8% positive PT reactions with ylang-ylang oil (5% pet.) (14). The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with ylang-ylang oil (2% pet.) 13.4% positive reactions (9). The Belsito 2006 study (20) yielded 0.6% positive reactions to ylang-ylang oil. The subsequent NACDG 2009 study identified 1.5% positive reactions in 4434 patients PTed with 2% “ylang-ylang oil” (21). The IVDK 2010c study found 2.5% positive reactions in 3175 consecutively tested patients, and 3.9% in 2155 patients tested in the context of a special series (30). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=12 reacted positively to ylang-ylang oil and 3 to “cananga oil” (48).

Cananga oil

ISO 4720:2009 nomenclature: *Cananga odorata* (Lam.) Hook. f. et Thomson *forma macrophylla*. For Oil of cananga (*Cananga odorata* (Lam.) Hook. f. et Thomson, *forma macrophylla*) an ISO standard exists: ISO 3523:2002. Cananga oil is produced by steam distillation of the flowers of *Cananga odorata* (DC.) Hook f. et Thomson subsp. *macrophylla* (*Annonaceae*). The composition resembles that of “ylang-ylang III”, but with a higher content of caryophyllene (30-40%). Cananga oil originates almost exclusively in Java; annual production about 50 t. The oil is used mainly in perfuming soaps where it is more stable than ylang-ylang oils due to its lower ester content (34).

Sugiura et al. (2000) found 1.1% positive reactions to “cananga oil”, tested 5% pet. (14). Cananga oil (2% pet.) mentioned in the same Portuguese study already cited (9) yielded 10.4% positive reactions. In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had positive reactions to cananga odorata oil tested at 2% concentration (13).

Studies with both oils

The Goossens 1997 study found 3 of 111 patients positive to “ylang-ylang oil 5% pet.”, and 4 to “cananga oil 15% pet.” – all sensitised to other fragrance allergens (23). The Rudzki 1976 study found 1 positive reaction in 200 patients to “cananga” and 4 to “ylang-ylang” essential oil, both tested 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=10 (11.6%) positive reactions to “cananga” and n=8 (9.3%) to “ylang-ylang” essential oils, each tested at 2% pet. (27). Nakayama et al. found 1974 (after (29)) 11 “strong positive” and 15 “weak positive” reactions to “Cananga oil” and 9 and 16, resp., to “Ylang-ylang oil” (unknown test concentration) in 183 patients.

A number of case reports highlight the possibility of occupational contact and sensitisation, e.g. (222, 224).

Additional information:

Ylang-ylang oil

The composition of this essential oil is defined by a standard: ISO 3063:2004. Ylang-ylang oils are obtained by steam distillation of freshly picked blossoms of *Cananga odorata* (DC.) Hook f. et Thomson subsp. *genuina* (*Annonaceae*). The oil is produced mainly in Madagascar and the Comoro islands. Four fractions are collected at progressively longer distillation times and are known as “extra”, “I”, “II” and “III”. The composition of the various oil fractions depends on the duration of distillation. The first fraction has the highest content of strongly odiferous constituents such as p-cresyl methyl ether (5-16%), methyl benzoate (4-9%), (-)-linalool (7-24%), benzyl acetate (5.5-17.5%), and geranyl acetate (2.5-14%). The other fractions contain increasing amounts of sesquiterpene hydrocarbons such as caryophyllene, germacrene-D, and (E,E)-alpha-farnesene (> 70% in “ylang-ylang III”). Components such as p-cresol, eugenol and isoeugenol are important for odour, although they are present only in low concentration (34). According to (30) the maximum observed concentration in ylang-ylang I and II are (in %): germacrene-D (28); (E,E)-alpha-farnesene (21); caryophyllene (17); linalool (I: 19.0; II: 9.5); benzyl benzoate (8.0); farnesol (4.0); benzyl salicylate (4.0); (E,E)-farnesyl acetate (3.5); geraniol (2.5); isoeugenol (0.8); benzyl alcohol (0.5); eugenol (0.5); p-cresyl methyl ether (I: 5.0; II 3.5); methyl benzoate (I: 5.5; II: 3.5); benzyl acetate (I: 10.0; II: 5.0); geranyl acetate (I: 15.0; II: 12.0).

CEDRUS ATLANTICA BARK OIL

CAS 92201-55-3; EC 295-985-9
(*Cedrus atlantica*, ext. = INCI) /
8000-27-9; EC / (Oils,
cedarwood) INCI name: CEDRUS
ATLANTICA OIL

Cedarwood oil

Current regulation: /

Clinical data:

In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=5 (0.7%) positive reactions to cedarwood oil 10% pet. (22). (The exact origin of “cedarwood oil” in this study is not clear.) The IVDK 2010 c study identified 0.8% positive reactions in 6223 patients tested in the context of a special series with a cedarwood oil tagged with CAS # 8000-27-9 (30).

Additional information:

Cedrus Atlantica Bark Oil is the volatile oil obtained from the bark of *Cedrus atlantica*, *Pinaceae*

(<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=55309>, last accessed 2010-01-05). The main odiferous component is alpha-atlantone [32207-08-2] (39)

Nomenclature also used: *Cedrus atlantica* wood oil (*Cedrus atlantica* (Endl.) G.Manetti ex Carrière)²²

See also *Juniperus virginiana*.

²² ISO 4720:2009 nomenclature

CEDRUS DEODARA WOOD OILCAS 91771-47-0; EC 294-939-5 (*Cedrus deodara*, ext.)*Cedarwood oil*

Current regulation: /

Clinical data:

The Rudzki 1976 study found 3 positive reactions in 200 patients to “cedarwood” essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to “Himalayan cedarwood” essential oil 2% pet. (27). (The labelling in the latter report points to *Cedrus deodara* as source of “cedarwood oil” in these 2 Polish studies.)

Additional information:

Cedrus Deodara Wood Oil, Himalayan cedarwood oil (*Cedrus deodara* (Roxb. ex D.Don) G. Don)²³, is the volatile oil obtained by steam distillation of the stumps of the Deodar Cedar, *Cedrus deodara*, *Pinaceae* (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=55311>, last accessed 2010-01-29).

Several other conifer species are called cedars, and the corresponding oils vary considerably in composition. These include Cedar leaf oil (*Thuja* oil) produced by steam distillation of fresh leaves and branch ends of *Thuja occidentalis* L. (*Cupressaceae*) from North America, containing a minimum of 60% thujone [8007-20-3] [90131-58-1] (34). Texas cedarwood oil is produced by steam distillation of chopped wood of *Juniperus mexicana* Schiede (*Cupressaceae*), containing alpha-cedrene (15-25%), thujopsene (25-35%), cedrol 20% minimum [8000-27-9] [91722-61-1] (34). Chinese cedarwood oil is similar to Texas cedarwood oil, obtained by steam distillation of *Cupressus funebris* Endl., *Cupressaceae* (*Chamaecyparis funebirs* (Endl.) France), which is a weeping cypress [8000-27-9] [85085-29-6] (34).

CINNAMOMUM CASSIA LEAF OIL

94961-46-6 [invalid] / 8007-80-5; EC / (Oils, cassia) INCI name: CINNAMONUM CASSIA OIL

*Cassia Oil; Cassia leaf Oil; Cinnamon Oil Chinense***CINNAMOMUM ZEYLANICUM BARK OIL**CAS 84649-98-9; EC 284-635-0 (*Cinnamomum zeylanicum*, ext. = INCI)*Cimmamon Bark Oil Ceylon; Cinnamon Oil Ceylon*

Current regulation: /

Clinical data:

The Rudzki 1976 study found 2 positive reactions in 200 patients to “cassia” essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=24

²³ ISO 4720:2009 nomenclature

(27.9%) positive reactions to "cassia" essential oil 2% pet. (27).

A 32 year old Spanish physiotherapist developed vesicular hand dermatitis after using a "balsam from ash extract" cream. PTing revealed positive reactions to this cream, the FM I, eugenol, and 2 components of the cream: "cinnamon oil" (0.5% pet.) and clove oil (1% pet.) (225).

Additional information:

ISO 4720:2009 nomenclature: *Cinnamomum tsumu* Helms, syn. *Cinnamomum cassia* auct. and *Cinnamomum zeylanicum* Blume syn. *Cinnamomum verum* J. Presl, respectively. Cassia oil (Chinese cinnamon oil) is obtained by steam distillation of the leaves, twigs, and bark of *Cinnamomum aromaticum* Nees (*C. cassia* Blume, *Lauraceae*). In contrast to cinnamon bark oil (see below), cassia oil contains a considerable amount of 2-methoxycinnamal (3-15%), in addition to its main constituent, cinnamal (70-88%). Cassia oil is predominantly used in flavouring soft drinks, with an annual production of a few hundred tons (34). For Oil of cassia, Chinese type (*Cinnamomum aromaticum* Nees, syn. *Cinnamomum cassia* Nees ex Blume) an ISO standard exists: ISO 3216:1997

Cinnamomum Zeylanicum Bark Oil is the volatile oil expressed from the bark of the Ceylon Cinnamon, *Cinnamomum zeylanicum*, *Lauraceae*. It contains mainly cinnamaldehyde (34), e.g. 50-60%, and lesser quantities of eugenol (4-8%), phellandrene

(<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=75370>, last accessed 2009-11-16). For Oil of cinnamon leaf, Sri Lanka type (*Cinnamomum zeylanicum* Blume) an ISO standard exists: ISO 3524:2003

Cinnamomum Cassia Leaf Oil is the volatile oil obtained by steam distillation from the leaves and twigs of the Chinese Cinnamon, *Cinnamomum cassia* (L.), *Lauraceae*. It contains 80% eugenol (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=75368>, last accessed 2009-11-16). The cinnamon leaf oil produced by steam distillation of the leaves of *Cinnamomum zeylanicum* Blume (*C. verum* J.S. Presl) similarly has a content of 70-83% eugenol (34).

Considering the content of well-known allergenic compounds, the essential oil is considered an Established contact allergen in humans,

CITRUS AURANTIUM AMARA FLOWER OIL

CAS 8016-38-4, 68916-04-1; EC / (Oils, neroli) /

Neroli oil

CITRUS AURANTIUM AMARA PEEL OIL
EXPRESSED

72968-50-4; EC 277-143-2 (Orange, sour, ext.)

"Bitter Orange Oil"

INCI names: CITRUS AURANTIUM AMARA ...

Current regulation: /

Clinical data:

The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "neroli oil" (2% pet.) 6.6% positive reactions (9). The Rudzki 1976 study found 3 positive reactions in 200 patients to "bitter orange" essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=2 (2.3%)

positive reactions to “bitter orange” essential oil 2% pet. (27). The IVDK 2010 c study identified 0.7% positive reactions in 6220 patients tested in the context of a special series (30)

Additional information:

ISO 4720:2009 nomenclature: *Citrus aurantium* L., syn. *Citrus amara* Link, syn. *Citrus bigaradia* Loisel, syn. *Citrus vulgaris* Risso. For Oil of neroli (*Citrus aurantium* L. spp. *aurantium*, syn. *Citrus aurantium* L. spp. *amara* var. *pumilia*) an ISO standard exists: ISO 3517:2002. Citrus Aurantium Peel Oil Expressed is an essential oil expressed from the fresh epicarps of the Sour Orange, *Citrus aurantium*, Rutaceae. It contains D-limonene (about 90%), citral, decanaldehyde, methyl anthranilate, linalool, terpineol (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41394>, last accessed 2010-01-29). The aldehyde content is lower and the ester content (e.g., linalyl and geranyl acetate) is higher than in sweet orange oil (34). It is predominantly used for flavouring alcoholic beverages. According to (30) the maximum observed concentration in neroli oil are (in %): linalool (44); limonene (18); β -pinene (17); linalyl acetate (15); *trans*- β -ocimene (8); geranyl acetate (5); *trans*-nerolidol (5); (*E,E*)-farnesol (4); myrcene (4); farnesol (4,0); geraniol (3,5); citral (0,3) (30).

CITRUS AURANTIUM AMARA LEAF OIL

72968-50-4; EC 277-143-2 (Orange, sour, ext.)

Petitgrain oil Paraguay / ... bigarade

Current regulation: /

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to “Petitgrain bigarade” and “Petitgrain Paraguay” essential oil each, both tested at 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=7 (8.1%) positive reactions to “Petitgrain bigarade” and n=4 (4.6%) to “Petitgrain Paraguay” essential oil each, both tested at 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Citrus sinensis* L. Pers. X *Citrus aurantium* L. ssp. *amara* var. *pumilia*. Petitgrain oils in general are steam distilled from the leaves of citrus trees. Citrus Aurantium Leaf Oil is an essential oil obtained from the leaves of the Sour Orange, *Citrus aurantium*, Rutaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41392>, last accessed 2010-02-10). Petitgrain oil Paraguay is obtained from an acclimatised variety of the bitter orange tree. Main constituents are linalool (15-30%) and linalyl acetate (40-60%). A number of trace constituents contribute essentially to the odour (34). Petitgrain oil bigarade is derived from the same species of tree grown in France, Italy, Spain and North Africa (34). For Oil of bitter orange petitgrain, cultivated (*Citrus aurantium* L.) an ISO standard exists: ISO 8901:2003.

Considering the content of well-known allergenic compounds, the essential oil is regarded as an established contact allergen in humans

CITRUS BERGAMIA PEEL OIL EXPRESSED

CAS 89957-91-5, 8007-75-8; EC

	289-612-9 (<i>Bergamot, ext.</i>)
<i>Bergamot Oil, Bergamot Orange Oil</i>	INCI: CITRUS AURANTIUM BERGAMIA EXTRACT

Current regulation: /

Clinical data:

The Rudzki 1976 study found 3 positive reactions in 200 patients to “Bergamot” essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found no positive reaction to “Bergamot” essential oil 2% pet. (27). In 63 patients positive to the FM I, 2 had a positive PT reaction to bergamot oil, 2% pet., in the Santucci 1987 study (28). A case report from Zacher and Ippen describes 2 patients with allergic contact dermatitis due to bergamot oil (191), one a worker in a perfume factory, the other sensitised by non-occupational use of cosmetics.

Additional information:

ISO 4720:2009 nomenclature: *Citrus bergamia* (Risso et Poit.), syn. *Citrus aurantium* L. subsp. *bergamia* (Wight et Arnott) Engler. Citrus Bergamia Peel Oil Expressed is an essential oil expressed from the epicarps of the Bergamot, *Citrus bergamia* risso, Rutaceae. It contains 35-45% L-linalyl acetate, about 6% linalool, D-limonene, DL-limonene and bergaptene (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=se_arch.details&id=41398, last accessed 2009-11-27). According to Surburg/Panten: linalyl acetate 22-36%, linalool 3-15%, geranial 0.25-0.5%, citral 1%, with a relatively low terpene content of 25-50% (34, 39). Bergaptene content by HPLC is 0.18-0.38% (34). Annual production from Italy, Brazil, Spain and Ivory Coast is 100 to 150 t. For Oil of bergamot [*Citrus aurantium* L. subsp. *bergamia* (Wight et Arnott) Engler], Italian type an ISO standard exists: ISO 3520:1998.

CITRUS LIMONUM PEEL OIL EXPRESSED	CAS 84929-31-7, 8008-56-8; EC 284-515-8 (<i>Lemon, ext.</i>)
<i>Lemon oil</i>	INCI names: CITRUS MEDICA LIMONUM ...

Current regulation: /

Clinical data:

The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with “lemon oil” (2% pet.) 4.5% positive reactions (9). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=2 (0.3%) positive reactions to “lemon oil” 2% pet. (22).

The Rudzki 1976 study found 1 positive reaction in 200 patients to “lemon” essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=2 (2.3%) positive reactions to “lemon” essential oil 2% pet. (27). The IVDK 2010 c study identified 0.3% positive reactions in 6467 patients tested in the context of a special series (30).

Additional information:

ISO 4720:2009 nomenclature: *Citrus limon* (L.) Burm. f. According to (30) the maximum observed concentration in lemon oil are (in %): limonene (80); β -pinene (16.5); γ -terpinene (12); citral (3.0); geranial (2.0); neral (1.2); β -bisabolene (0.9); geranyl

acetate (0.7); neryl acetate (0.6); linalool (0,3); geraniol (0,2) (30). An ISO standard exists for Oil of lemon [Citrus limon (L.) Burm. f.], obtained by expression: ISO 855:2003. The composition of lemon oil depends on the variety of lemon and the country of origin, see table from (34).

Table 3. Specifications for qualities of lemon oils of different origins

Parameter	Type	Mediterranean		Equatorial	
	American Origin				
	Coast	Desert	Italy	Spain	Ivory coast, Brazil
d_{20}^{20}	0.851–0.857	0.849–0.854	0.850–0.858	0.849–0.858	0.845–0.854
n_D^{20}	1.4370–1.4760	1.4370–1.4760	1.4370–1.4760	1.4370–1.4760	1.4370–1.4790
α_D^{20}	+57° to +65° 6'	+67° to +78°	+57° to +66°	+57° to +66°	+57° to +70°
Composition by GC [area %]					
β -Pinene	9–14	10–13	10–16.5	10–16.5	7–16
Limonene	63–70	70–80	60–68	60–70	59–75
γ -Terpinene	8.3–9.5	6.5–8	8–12	8–12.8–12	6–12
Neral	0.6–0.9	0.3–0.6	0.6–1.2	0.4–1	0.2–1.2
Geraniol	1.0–2	0.5–0.9	0.8–2	0.6–2	0.5–2
Evaporation residue [weight %]					
	1.75–3.9		1.5–3.9	1.5–3.9	1.5–4
Carbonyl value					
	8–14	6.25–12	11–17	11–17	6–17
CD value	min. 0.2	min. 0.2	0.45–0.9	0.4–0.9	0.2–0.96

CITRUS PARADISI PEEL OIL

CAS 8016-20-4 ; EC /

Grapefruit oil, expressed

INCI: CITRUS GRANDIS OIL

Current regulation: II/358 R1

Clinical data: /

Additional information:

Citrus Paradisi Peel Oil is the volatile oil expressed from the peel of the Grapefruit, Citrus paradisi, Rutaceae
http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=55434

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

CITRUS SINENSIS (syn.: AURANTIUM DULCIS) CAS 97766-30-8, 8008-57-9, EC
 PEEL OIL EXPRESSED 307-891-8 (Orange, sweet,

(Sweet) Orange oil

Valencia, ext. = INCI) / 8028-48-6; EC 232-433-8 (Orange, sweet, ext.)

INCI names: CITRUS AURANTIUM DULCIS ...

Current regulation: /

Clinical data:

The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "orange oil" (2% pet.) 4.5% positive reactions (9). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=1 (0.1%) positive reactions to orange oil 2% pet. (22). The Rudzki 1976 study found 1 positive reaction in 200 patients to "sweet orange" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to "sweet orange" essential oil 2% pet. (27). In the Frosch 1995 dose-finding pilot study, neither positive nor irritant reaction to 1% and 5% "orange oil Bras." in pet., tested in 205 consecutive patients in Dortmund and Göttingen, were observed (15). The IVDK 2010 c study identified 0.2% positive reactions in 6246 patients tested in the context of a special series (30).

Additional information:

ISO 4720:2009 nomenclature: *Citrus sinensis* (L.) Osbeck. For Oil of sweet orange (*Citrus sinensis* (L.) Osbeck), CAS 8008-57-9, obtained by mechanical treatment, an ISO norm exists: ISO 3140:2005. The oils have a high terpene hydrocarbon content (> 90%, mainly (+)-limonene. Important for aroma are aldehydes, mainly decanal and citral, and aliphatic and terpenoid esters. The sesquiterpene aldehydes alpha-sinensal [17909-77-2] and beta-sinensal [6066-88-8] contribute particularly to the special sweet aroma (34). According to (30) the maximum observed concentration in sweet orange oil are (in %): *limonene* (95.0); *linalool* (0.7); n-decanal (0.7); *citral* (0.3); alpha-sinensal (0.05); beta-sinensal (0.06) (30). Worldwide production is more than 30000 tons / year. Main uses comprise the flavouring of beverages and confectioneries and perfuming E.d.C, soaps and household products.

For the latter uses relevant here, both "Orange peel oil, sweet (*Citrus sinensis* (L.) Osbeck) (8008-57-9)", "Orange peel, sweet, extract (*Citrus sinensis* L. Osbeck) (8028-48-6)" and "Orange, sweet, Valencia, ext. (97766-30-8)" are among the top 100 used fragrance materials and classified as R43 (IFRA, pers. comm. 2010).

ORANGE OIL TERPENES (CAS # 68647-72-3) are a "top 100 mixture of substances and classified as R43 (IFRA, pers. comm.2010). Other names: ORANGE, SWEET, TERPENES (REACH); Terpenes and Terpenoids, sweet orange-oil (REACH). The CAS entry refers to a group of substances "Terpenes and Terpenoids, sweet orange-oil" (REACH).

CITRUS TANGERINA ...

Oil of tangerine

CAS 223748-44-5; EC /

[no info in CAS database]

Current regulation: /

Clinical data:

In a 17 year old girl, the perfume used for 3 months caused ACD due to the ingredient "oil of tangerine", with a strong positive PT reaction (to 2% or 10% in pet.; 50 controls

negative) (226).

Additional information:

Citrus Tangerina Peel Oil is the volatile oil expressed from the peel of the ripe fruit the Tangerine, *Citrus Tangerina*, *Rutaceae* (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=55441>, last accessed 2010-01-29); (*Citrus tangerina* Tanaka).

CORIANDRUM SATIVUM HERB OIL

CAS 84775-50-8; EC 283-880-0
(Coriander, ext.)

Coriander oil

INCI: CORIANDRUM SATIVUM
EXTRACT

Current regulation: /

Clinical data:

The Rudzki 1976 study found 2 positive reactions in 200 patients to “coriander” essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to “coriander” essential oil 2% pet. (27).

Additional information:

Coriander Sativum Herb Oil is an essential oil obtained from the herbs of the Coriander, *Coriandrum sativum* L., *Umbelliferae* (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39388>, last accessed 2010-01-29). The main component of coriander oil is linalool (by GC: 65-78%) and mono- and polyunsaturated fatty aldehydes contributing to the particular aroma. In contrast to the seed oil, coriander leaf oil contains these aldehydes as main constituents, e.g. 2-deccanal and 2-dodecanal (34). For Oil of coriander fruits (*Coriandrum sativum* L.) an ISO standard exists: ISO 3516:1997.

CYMBOPOGON OILS

Cymbopogon oils are produced from several aromatic grasses that belong to the genus *Cymbopogon* Speng. (*Poaceae*). The oils are obtained by steam distillation of the aerial parts of the plants (34).

The composition of the essential oil derived from *Cymbopogon flexuosus* (Nees ex Steudel) J.F. Watson is defined by a standard: ISO 4718:2004, as is the oil derived from *Cymbopogon citratus*: 3217:1974.

CYMBOPOGON CITRATUS LEAF OIL

Cymbopogon citratus (DC.) Stapf.²⁴

CAS 89998-14-1; EC 289-752-0
(*Cymbopogon citratus*, ext. =
INCI)

²⁴ ISO 4720:2009 nomenclature

Lemon Grass Oil; Indian Verbena Oil; Indian Melissa Oil

CYMOPOGON SCHOENANTHUS OIL

Cymbopogon flexuosus (Nees ex Steudel) J.F. Watson²⁵

CAS 8007-02-1; EC 289-754-1 (oils, lemongrass) / 89998-16-3; EC 289-752-0 (Cymbopogon Schoenanthus, ext. = INCI)

Lemon Grass Oil

Current regulation: /

Clinical data:

The Frosch 2002 b study on 1606 consecutive patients reported 1.6% positive reactions to "lemongrass oil (East India), CAS 8007-02-1", PTed at 2% pet. (17). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 3 positive reactions to lemongrass oil were observed (3). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=6 (0.8%) positive reactions to lemongrass oil 2% pet. (22). The IVDK 2010 c study identified 0.6% positive reactions in 2435 consecutively tested patients and 2.3% positive reactions in 8445 patients tested in the context of a special series (30).

Additional information:

Cymbopogon Citratus Leaf Oil is an essential oil obtained from the leaves of the Lemon Grass, *Cymbopogon citratus* (DC., ex Nees), *Poaceae*. It contains citral (75-85%), methylheptenone, citronellal, geraniol, limonene (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39457>, last accessed 2009-11-12). According to Surburg/Panten, by GC: neral (31-40%), geranial (40-50%) (34).

Indian lemongrass oil is obtained by the so-called Indian variety of lemongrass, *Cymbopogon flexuosus* (Nees ex Steud.) Stapf. Content by GC: 25-35% neral, 35-47% geranial (34).

Cymbopogon Schoenanthus Oil is the volatile oil obtained by the steam distillation of fresh Lemon Grass, *Cymbopogon schoenanthus* (L.), *Poaceae* (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=75419>, last accessed 2009-11-12). According to (30) the maximum observed concentration in lemongrass oil are (in %): citral (85.0); geraniol (7.0); limonene (4.0); geranyl acetate (2.2); caryophyllene (1.6); trans-isocitral (1.4); 6-methyl 5-hepten-2-one (1.3); caryophyllene oxide (1.2); 4-nonanone (1); citronellol (0.8); eugenol (0.3); linalool (0.2) (also according to (227))

In a LLNA study by RIFM, the lemongrass oil as used was reported to contain 68.8% citral, 6.7% limonene, 6.1% geraniol, 2.2% geranyl acetate, 1.6% caryophyllene, 1.4% trans-isocitral, 1.3% 6-methyl 5-hepten-2-one, 1.2% caryophyllene oxide and 1% 4-nonanone, according to analyses of the supplier. The EC3 value was calculated to be 6.5% (227).

CYMOPOGON MARTINI HERB EXTRACT

CAS 84649-81-0; EC 283-461-2 (Cymbopogon Martini, ext)

²⁵ ISO 4720:2009 nomenclature

INCI: CYMBOPOGON MARTINI OIL*Palmarosa oil*

Current regulation: /

Clinical data: /

Additional information:

ISO 4720:2009 nomenclature: *Cymbopogon martini* (Roxb.) Will. Watson var. *motia* and var. *sofia*. Cymbopogon Martini Herb Extract is an extract obtained from the herbs of the plant, *Cymbopogon martini*, Gramineae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39460>, last accessed 2009-11-24), namely, by steam distillation of wild or cultivated *Cymbopogon martini* (Roxb.) J.F. Wats., collected when in blossom (34). The main constituent is geraniol (72-94%) (34).

In a LLNA study by RIFM, the palmarosa oil as used was reported to contain 79.4% geraniol, 9.4% geranyl acetate and 1.9% caryophyllene, according to analyses of the supplier. The EC3 value was calculated to be 9.6% (227).

CYMBOPOGON NARDUS HERB OIL

CAS 89998-15-2; EC 289-753-6 (*Cymbopogon nardus*, ext. = INCI)

Citronella Oil (Sri Lanka)

CYMBOPOGON WINTERIANUS HERB OIL

CAS 91771-61-8; EC 294-954-7 (*Cymbopogon Winterianus*, ext. = INCI)

Citronella Oil (Java)

Current regulation: ...

Clinical data:

The Rudzki 1976 study found 5 positive reactions in 200 patients to "citronella" essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=1 (1.1%) positive reactions to "citronella" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Cymbopogon nardus* (L.) W. Watson var. *lenabatu* Stapf. and *Cymbopogon winterianus* Jowitt, respectively. Cymbopogon Nardus Herb Oil is an essential oil obtained from the herbs of the plant, *Cymbopogon* (syn: *Andropogon*) *nardus* (L.), Gramineae. The Ceylon citronella oil contains geraniol (about 60%), citronellal (about 15%), camphene, limonene, linalool, borneol. According to Surburg/Panten, the Sri Lankan oil contains citronellal (3-6%), borneol (4-7%), citronellol (3-8.5%), geraniol 15-23%) and methyl isoeugenol (7.11%) (34).

The Java citronella oil contains 25-50% citronellal, 25-45% geraniol (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.h.details&id=39469>, last accessed 2009-11-24). Cymbopogon Winterianus Herb Oil as a synonym for Java citronella oil is obtained from the herbs of the plant, *Cymbopogon winterianus*, Gramineae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=sea>

[rch.details&id=39472](#), last accessed 2009-11-24). This oil, produced in Taiwan and Java, contains citronellal (31-40%), geraniol (20-25%), citronellol (8.5-14%), geranyl acetate (2.5-5.5%), citronellyl acetate (2-4%) and many minor components. Annual worldwide production is currently at around 1000 t (34). For Oil of citronella, Sri Lankan type (*Cymbopogon nardus* (L.) W. Watson var. *lenabatu* Stapf.) an ISO standard exists: ISO 3849:2003, for Oil of citronella, Java type the ISO 3848:2001.

In a LLNA study by RIFM, the citronella oil as used was reported to contain 36.6% citronellal, 20.6% geraniol, 4.1% limonene, 3.7% geranyl acetate, 3.0% citronellyl acetate, 2.6% elemol, 2.2% beta-bourbonene, 1.9% delta-cadiene, 1.6% isopugenol I, 1.4% germacrene D and eugenol and linalol at < 1%, according to analyses of the supplier. The EC3 value was calculated as > 50 % (227).

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

EUCALYPTUS SPP. LEAF OIL

CAS 92502-70-0; EC 296-357-7
(*Eucalyptus*, ext. = INCI)

Eucalyptus Oil

CAS 8000-48-4; EC / (Oils,
eucalyptus) INCI: *EUCALYPTUS*
GLOBULUS OIL

Current regulation: /

Clinical data:

In a study with 218 fragrance sensitive patients, 1.8% reacted positively to 10% eucalyptus oil (pet.) (1). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 1 positive reaction to "eucalyptus oil" was observed (3). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=4 (0.6%) positive reactions to eucalyptus oil 2% pet. (22). The Rudzki 1976 study found 3 positive reactions in 200 patients to "eucalyptus" essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=1 (1.1%) positive reactions to "Eucalyptus" essential oil 2% pet. (27). The IVDK 2010 c study identified 0.2% positive reactions in 6680 patients tested in the context of a special series (30).

In a professional athlete, the use of an "analgesic and anti-inflammatory cream" over 2 years lead to ACD, which was attributed to eucalyptol (eucalyptus oil, 1% pet., 25 controls negative), the sole ingredient of the cream eliciting a positive PT reaction (228)

Additional information:

ISO 4720:2009 nomenclature: *Eucalyptus globulus* Labill. Eucalyptus oils are produced from plants belonging to the genus *Eucalyptus* (*Myrtaceae*), which includes about 500 species in Australia, the country of origin, alone. At present, few of the oils, which are used to characterise species, are commercially important (34). Some species are rich in 1,8-cineole (80-85% content). Other species contain less cineole, but 10-22% alpha-pinene. *E. citriodora* predominantly contains citronellal (min. 75% by GC), with some citronellol and isopulegol (5-10% each) (34). *E. dives* contains (-)-piperitone and 15-25% alpha-phellandrene (34). According to (30) the maximum observed concentration in eucalyptus oil are (in %): 1,8-cineole (58; 70-80 after rectification); α -pinene (22); limonene (8); para-cymene (5); trans-pinocarveol (5); aromadendrene (10); globulol (2.5) [the latter 2 components only traces after rectification] (30).

For Crude or rectified oils of *Eucalyptus globulus* (*Eucalyptus globulus* Labill.) an ISO standard exists: ISO 770:2002.

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

EUGENIA CARYOPHYLLUS LEAF / FLOWER OIL

CAS 8000-34-8; EC / (Oils, clove)

Clove oil

INCI: EUGENIA CARYOPHYLLUS OIL

Current regulation: /

Clinical data:

In the Larsen 2002 c study, 19.3% of patients with known contact allergy to fragrance ingredients reacted positively to "clove bud oil" (10 % pet.) (1). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 2 positive reactions to "clove oil" were observed (3). The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with clove oil (2% pet.) 13.4% positive reactions (9). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 1.6% positive reactions 2% pet. (22). The Rudzki 1976 study found 2 positive reactions in 200 patients to "clove" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=12 (13.3%) positive reactions to "clove" essential oil 2% pet. (27). The IVDK 2010 c study identified 1.5% positive reactions 6893 patients tested in the context of a special series (30).

A 32 year old Spanish physiotherapists developed vesicular hand dermatitis after using a "balsam from ash extract" cream. PTing revealed positive reactions to this cream, the FM I, eugenol, and 2 components of the cream: cinnamon oil (0.5% pet.) and clove oil (1% pet.) (225).

Additional information:

ISO 4720:2009 nomenclature: *Syzygium aromaticum* (L.) Merr. & L. M. Perry syn. *Eugenia caryophyllus* (Spreng.) Bullock & S. G. Harrison. Standards regarding the composition of clove oil are available: ISO 3141:1997, ISO 3142:1997, ISO 3143:1997. Clove oils are produced from the clove tree *Syzygium aromaticum* (L.) Merr. et L.M. Perry [*Eugenia caryophyllus* (Spreng.) Bullock ex S.G. Harrison]. The content of clove bud, clove leaf and clove stem oil has, with little variation, been determined by GC as 75-92% eugenol, 2-17% caryophyllene and 0.2-15% eugenyl acetate – the latter compound found in particularly high concentration in bud oil (34). According to another source, the following maximum content (%) has been observed regarding the constituents listed: eugenol (92,0);

caryophyllene (17); eugenyl acetate (15); isoeugenol (0.5) (30).

In a LLNA study by RIFM, the clove leaf oil as used was reported to contain 85.3% eugenol, 9.9% caryophyllene and 2.2% alpha humulene, according to analyses of the supplier. The EC3 value was calculated to be 7.1% (227).

EVERNIA FURFURACEA LICHEN EXTRACT

CAS 90028-67-4; EC 289-860-8
(*Evernia furfuracea*, ext. = INCI)

Tree moss extract

Current regulation: /

Clinical data:

The Larsen 1977 study in 20 “perfume-sensitive patients” yielded n=6 positive reactions with “treemoss abs. in benzyl benzoate, 5% petrolatum” (18). In the IVDK 2007 study, 2.7% (95% CI: 2.0 – 3.6%) of 1658 consecutive patients had a positive reaction to “tree moss absolute” (4). In the Groningen 2009 study, 2.5% (95% CI: 1.1 – 4.9%) had positive reactions to the allergen, tested at 2%, i.e., twice the commonly used concentration, and not in pet., but in diethylphthalate (6). The IVDK 2010 study, 6.02% (95% CI: 4.90 – 7.14%; percentages standardised for age and sex) of 1947 patients PTed reacted to the compound (7).

Additional information:

Syn.: *Pseudoevernia furfuracea* (L.) Zopf (53). The lichen grows on the bark of pine and fir trees. The extraction process with carbohydrate solvents yields a “concrete” (2-5% yield) which, in a next step eliminating waxy compounds, is extracted with warm alcohol and subsequent cooling, yielding an “absolute” (40-60% yield) (53).

EVERNIA PRUNASTRI

CAS 90028-68-5; EC 289-861-3
(*Evernia prunastri*, ext. = INCI)

Oak moss abs.

Current regulation: Annex III, part 1, n° 91

Clinical data:
In the “background information” section of the 1999 opinion, oak moss extract is classified as “most frequently reported allergen”; in consecutive PT patients, about 2.8% positive reactions had been reported (33). The German MAK commission has labelled oak moss extract as ‘sensitising to the skin’ (229).

Since the last SCCNFP-opinion of 1999, a “polymer based method” was developed to reduce the natural content of these two compounds from around 1 - several percent to < 75 ppm for atranol and < 25 ppm for chloratranol. However, PTing 14 subjects with previous positive PT reactions to the “oak moss” allergen preparation with the modified *Evernia prunastri* material still elicited positive reactions in 8/14 subjects; thus, the reduction in allergen content was deemed unsafe for the consumer (230). In a study of 885 consecutive eczema patients tested in Gentofte, Denmark, 3.2% had a positive or follicular patch test response to oak moss absolute. Two types of oak moss absolute were tested, one contaminated by resin acids and one without any detectable resin acids. There was no difference in reactivity between the two types of oak moss absolute

(231). The IVDK 2007 study yielded 2.2% (95% CI: 1.6 – 3.0%) positive reactions in 2063 consecutively tested patients (4). In the Groningen 2009 study, 1.9% (95% CI: 0.7 – 4.0%) had positive reactions to oak moss, tested at 2% pet., i.e., twice the commonly used concentration (6). In the An 2005 study, 6 of 422 consecutive patients, i.e., 1.4%, had positive reaction (13) (test concentration 2% pet.). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 5.0% positive reactions (22). The IVDK 2010 study, 1.81% (95% CI: 1.07 – 2.56%) of 1213 consecutively tested patients reacted to the compound, while 5.59% (95% CI: 4.90 – 6.27%) of 4482 of patients tested in a more aimed manner, partly as breakdown testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were tested with *E. prunastri* extract, yielding 2 positive reactions (48).

L. Kanerva et al. report on a 41 year old female hairdresser in whom oak moss abs. contained in a perming solution (concentration in the product unknown) was unequivocally identified as allergen causing (i) occupational hand dermatitis and (ii) scalp dermatitis after application to the own hair (232). Another case of occupational hand dermatitis in a grinding engineer was, at least partly, attributable to contact sensitisation to "oak moss resin" contained in a soluble oil (233).

Additional information:

Source: *Evernia prunastri* (Oak moss) (*Evernia prunastri* var. *prunastri* L. Ach). Oak moss is extracted as described above. Chloratranol and atranol are the degradation products of chloratranorin and atranorin, resp., which are recognised as the main sensitisers in *Evernia prunastri* extracts.

ILLICIIUM VERUM FRUIT OIL

CAS 84650-59-9, 8007-70-3; EC 283-518-1

"Anise Oil", Star anise oil

(Star anise, *Illicium verum*, ext. = INCI)

Current regulation: /

Clinical data:

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

Additional information:

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

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

Current regulation: /

Clinical data:

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

Additional information:

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

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

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

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

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

JUNIPERUS VIRGINIANA OIL

CAS 8000-27-9; EC / (Oils, cedarwood) [this also refers to *Cedrus atlantica* ...] / 85085-41-2; EC 285-370-3 (Juniper, *Juniperus virginiana*, ext. = INCI)

JUNIPERUS VIRGINIANA WOOD OIL

CAS 85085-41-2; EC 285-370-3

Cedar Wood Oil (Virginian)

Current regulation: /

Clinical data:

In the Frosch 2002 b study, a total of 0.6% of 1606 consecutive patients had a positive PT to "cedarwood oil (Moroccan and Chinese 1:1)", tested 10% in pet. (17). After application of Penaten-baby™ oil as immersion oil for dermatoscopy a patient developed multiple patches of eczema at the application sites. Investigation revealed that the oil was kept in a bottle previously used for *Juniperus virginiana* oil, to which contact sensitisation was verified by patch testing (235).

Additional information:

ISO 4720:2009 nomenclature: *Juniperus virginiana* L.. *Juniperus Virginiana* Oil is the volatile oil obtained from the fruits and leaves of the Red Cedar, *Juniperus virginiana* L., Cupressaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=78070>, last accessed 2010-01-05)

Juniperus Virginiana Wood Oil is an essential oil obtained from the wood and twigs of the Red Cedar, *Juniperus virginiana* L., Cupressaceae. It contains chiefly (alpha and beta) cedrene and cedral (cedar camphor), cuparene, thujopsene, widdrol (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39767>, last accessed 2009-11-12)(235). According to Surburg/Panten by GC: alpha-cedrene 22-35%, thujopsene 10-25%, cedrol 16-25% (34).

See also *Cedrus atlantica*. According to (30) the maximum observed concentration in cedar wood oil are (in %): α-cedrene (32); thujopsene (25); cedrol (25); β-cedrene (6); widdrol (5) and cuparene (traces) (30).

For Oil of cedarwood, Virginian (*Juniperus virginiana* L.) an ISO standard is available: ISO 4724:2004. For Oil of cedarwood, Texas (*Juniperus mexicana* Schiede) an ISO standard exists: ISO 4725:2004.

LAURUS NOBILIS OIL

CAS 8002-41-3; EC / (Oils, laurel)
 INCI: LAURUS NOBILIS OIL /
 8007-48-5; EC / (Oils, sweet
 bay)/ 84603-73-6; EC 283-272-5
 (Laurus nobilis, ext.) INCI:
 LAURUS NOBILIS EXTRACT

Laurel oil

Current regulation: Annex II, n° 359 (seed oil)

Clinical data:

In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=4 (0.6%) positive reactions to "laurel oil" 2% pet. (22).

After sensitisation by a one-time occlusive application a 36 year old Turkish patient developed widespread allergic contact dermatitis 3 days after massage with olive oil containing Laurus nobilis oil; sensitisation was proven by a strong positive reaction to the commercial test preparation and the massage oil previously used (236). Topical application of laurel oil for knee arthropathy led to an erythema exudativum multiforme-like rash on the legs of a 63 year old patient; interestingly, laurel oil yielded a "target like" strongly positive PT reaction in this case (237). In an earlier Turkish case with a similar history, the EEM-like appearance was lacking; however, a very intense, edematous reaction was noted (238). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 2 positive reactions to "laurel oil" were observed (3). The IVDK 2010 c study identified 1.0% positive reactions in 6297 patients tested in the context of a special series (30).

Additional information:

ISO 4720:2009 nomenclature: *Laurus nobilis* L. Laurel leaf oil is obtained by steam distillation of leaves from *Laurus nobilis* L. (Lauraceae), an evergreen cultivated primarily in the Mediterranean countries. The main components are 1,8-cineole (30-70%), linalool (about 10%) and eugenol (34). According to (30) the maximum observed concentration in laurel oil are (in %): 1,8-cineole (70); β -caryophyllene (11); linalool (11); limonene (5.0); eugenol (2.0); geraniol (0.3) (30).

LAVANDULA HYBRIDA HERB OIL

CAS 91722-69-9; EC 294-470-6
 (Lavender, *Lavandula hybrida*,
 ext. = INCI)

Lavandin Oil

Current regulation: /

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to "lavandin" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=4 (4.6%) positive reactions to "lavandin" essential oil 2% pet. (27). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% lavandin oil in pet., tested in 205 consecutive patients in Dortmund and Göttingen, and just 1 irritant reaction to

the higher concentration, were observed (15).

Additional information:

ISO 4720:2009 nomenclature: *Lavandula angustifolia* Mill. x *Lavandula latifolia* Medik. *Lavandula* *hybrida*, *Labiatae* (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39789>, last accessed 2010-01-29. Nomenclature according to Surburg/Panten: *Lavandula* x *intermedia* Lois, which is a hybrid of lavender and spike (see below) (34). The oils from the most important variants, abrial and grosso, contain linalool (24-38%), linalyl acetate (20-38%) as well as 1,8-cineole (4-11%), and camphor (6-11%) (34). A third variant is called super because of its high concentration of linalyl acetate (35-47%), more closely resembling lavender oil (34). For Oil of lavandin Grosso (*Lavandula angustifolia* Mill. x *Lavandula latifolia* Medik.), French type an ISO standard exists: ISO 8902:2009, for Oil of lavandin Abrial (*Lavandula angustifolia* Miller x *Lavandula latifolia* Medikus), French type a different ISO standard: ISO 3054:2001.

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

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

LAVANDULA OFFICINALIS FLOWER OIL

CAS 84776-65-8, 8000-28-0; EC 283-994-0 (*Lavender*, *Lavandula angustifolia angustifolia*, ext. = *INCI*)

Lavender oil

Current regulation: /

Clinical data:

In a large series from Nagoya, Japan, 1483 patients were tested with lavender oil 20% in pet., with overall 3.7% positive reactions from 1990 to 1998. However, within this period, a sharp increase was noted in 1997 and 1998, which as attributed to changed exposure by M. Sugiura et al. (14). On the individual level, relevance of positive reactions remained unclear in about half of the cases. The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "lavender absolute" (2% pet.) 6.6% positive reactions (9). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had positive reactions to "Lavandula augustifolia oil" (Lavender absolute) 2% (13). The Goossens 1997 study found 4 of 111 patients positive to "lavender oil 20% pet." – all of them sensitised to other fragrance allergens (23). The Rudzki 1976 study found no positive reaction in 200 patients to "lavender" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to "lavender" essential oil 2% pet. (27). Nakayama et al. found 1974 (after (29)) 6 "strong positive" reactions to "Lavender oil" (unknown test concentration) in 183 patients. In a study from Alicante, Spain, 86 selected patients were tested with "lavender absolute", yielding 2 positive reactions (48).

R. Goiriz et al. report on a case of photo contact allergy (10 controls negative) in a 45 year old woman developing after application of a ketoprofen-containing topical gel ("Fastum") (239). A physiotherapist developed acute, recurrent dermatitis after use of "Difflam® gel", scented with lavender oil. Both the gel and lavender oil (2% pet.) tested positive; avoidance resulted in clearing (240). In a study on 218 patients with known

contact allergy to fragrance ingredients, Larsen (2002 c) found positive reactions to 10% lavender oil (pet.) in 2.8% of these (1). A case of vulvovaginitis with spread and affecting the dominant hand applying various tea tree and lavender oil creams was reported by S. Varma; the PT with 10% lavender oil abs. in pet. (50 controls negative) was positive (241). In two cases, facial "pillow dermatitis" due to lavender oil, applied to the pillows, developed, confirmed by positive PT to lavender abs. (2% pet.) (242).

Additional information:

ISO 4720:2009 nomenclature: *Lavandula angustifolia* Mill. *Lavandula officinalis* Flower Oil is an essential oil obtained from the fresh flowering tops of the Lavender, *Lavandula officinalis* (syn: *L. vera*), *Labiatae*. It contains 30-40% esters calculated as linalyl acetate, linalool, pinene, limonene, geraniol, some eucalyptol (cineol) (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40370>, last accessed 2009-11-09). According to Surburg/Panten, lavender oil is obtained by steam distillation of freshly cut flowering tops of *Lavandula angustifolia* Mill. (Lamiaceae). Main constituents according to GC are linalyl acetate (25-45%), cis-ocimene (4-10%), trans-ocimene (1.5-6%), 1,8-cineole ($\leq 1\%$) camphor ($\leq 0.5\%$), linalool (25-38%), 1-terpinen-4-ol (2-6%) and lavandulyl acetate [25905-14-0] ($\geq 2\%$) (34).

In addition to distillation, both *Lavandula officinalis* and Lavandin are also solvent extracted, yielding concretes and, after ethanol extraction, absolutes, which are said to have a longer-lasting odour (34).

For Oil of lavender (*Lavandula angustifolia* Mill.) an ISO standard exists: ISO 3515:2002.

LAVANDULA SPICA HERB OIL

CAS 97722-12-8; EC 307-762-6
(Lavender, *Lavandula spica*, ext.
= INCI

"Spike Oil"

Current regulation: ...

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to "spike" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=8 (9.3%) positive reactions to "spike" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Lavandula latifolia* Medik. *Lavandula Spica* Herb Oil is an essential oil distilled from the flowering herbs of the Spikenard, *Lavandula spica* (syn: *Lavandula latifolia*), *Labiatae*. It contains eucalyptol (35%), camphor, linalool, borneol, terpineol, D-camphene and sesquiterpenes (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40372>, last accessed 2010-01-29). According to Surburg/Panten, Spanish spike lavender oil is steam distilled from the flowering tops of *Lavandula latifolia* Medik.. The main components are linalool (34-50%), 1,8-cineole (16-39%) and camphor (8-16%) (34). For Oil of spike lavender (*Lavandula latifolia* (L.f.) Medikus), Spanish type an ISO standard exists: ISO 4719:1999

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

LITSEA CUBEBA FRUIT EXTRACT

CAS 90063-59-5, 68855-99-2; EC 290-018-7 (*Litsea cubeba*, ext.)
INCI: LITSEA CUBEBA OIL

Current regulation: ...

Clinical data:

The Rudzki 1976 study found 3 positive reaction in 200 patients to “Litsea cubeba” essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=7 (8.1%) positive reactions to this essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Litsea cubeba* (Lour) Pers. Litsea Cubeba Fruit Extract is an extract obtained from the fruits of the plant, *Litsea cubeba*, Lauraceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40036>, last accessed 2009-11-24. The content by GC is: neral (25-33%), geranial (38-45%) – i.e. about ¾ citral, for which the extract had previously served as a raw material (34); direct use for perfuming is limited to household products (39). For Oil of Litsea cubeba (*Litsea cubeba* Pers.) an ISO standard exists: ISO 3214:2000.

In a LLNA study by RIFM, the “Litsea cubeba oil” as used was reported to contain 85.7% citral, 2.9% limonene, 1.7% linalool, 1.4% citronellal and < 1% caryophyllene and methyl heptanone, according to analyses of the supplier. The EC3 value was calculated as 8.4 % (227).

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

MENTHA ARVENSIS LEAF OIL

CAS 68917-18-0 ; EC /

Cornmint oil

INCI: MENTHA ARVENSIS OIL

Current regulation: /

Clinical data: /

Additional information:

Mentha Arvensis Leaf Oil is the oil derived from the leaves of the Horse Mint, *Mentha arvensis* L., Labiatae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=57860)

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

MENTHA PIPERITA OIL

CAS 8006-90-4; EC / (Oils, peppermint) INCI: MENTHA

*PIPERITA OIL / 84082-70-2; EC
282-015-4 (Peppermint, ext.) INCI
names: MENTHA PIPERITA ...*

Peppermint oil

Current regulation: /

Clinical data:

In the Frosch 2002 b study, 0.6% of 1606 consecutive patients reacted positively to "peppermint oil (American)", tested 2% in pet. (17). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 2 positive reactions to "peppermint oil" were observed (3). In the Wöhrle 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); menthofuran (8); isomenthone (8); neo menthol (6); pulegone (3.5); limonene (3.0); linalool (0.4) (30). Most of the safety test data concern Peppermint Oil. The oil is considered to present the "worst case scenario" because of its many constituents, so data on the oil were considered relevant to the entire group of ingredients. ... Repeated

intradermal dosing with Peppermint Oil produced moderate and severe reactions in rabbits” concluding that “with the limitation that the concentration of pulegone in these ingredients should not exceed 1%, it was concluded that Mentha Piperita (Peppermint) Oil, Mentha Piperita (Peppermint) Extract, Mentha Piperita (Peppermint) Leaves, Mentha Piperita (Peppermint) Water are safe as used in cosmetic formulations”.

MENTHA SPICATA HERB OIL

CAS 84696-51-5, 8008-79-5; EC 283-656-2 (Spearment, ext.)

Spearment oil

INCI: MENTHA VIRIDIS EXTRACT

Current regulation: /

Clinical data:

In the Frosch 2002 b study, 0.8% of 1606 consecutive patients reacted positively to “spearment oil (American)”, tested 2% in pet. (17). The CAS # quoted (8008-79-5) refers, according to CosIng, to MENTHA VIRIDIS LEAF OIL, the volatile oil obtained from the dried tops and leaves of the Garden Mint, *Mentha viridis* L., Labiatae. The Larsen 2001 study diagnosed 5.0% positive reactions in 178 patients with known contact allergy to fragrance ingredients, using this oil at 5% pet. test concentration (19). In the An 2005 study, 6 of 422 consecutive patients, i.e., 1.4%, had positive reactions to “*Mentha viridis* oil” 5% (13). PT results with toothpaste ingredients were positive in 7 patients, of whom 4 had strong positive reactions to spearment (246).

Additional information:

ISO 4720:2009 nomenclature: *Mentha spicata* L. *Mentha Spicata* Oil is an essential oil obtained from the herbs of the Spearment, *Mentha spicata* L., Labiatae (syn: *Mentha viridis* L., Labiatae). It contains carvone (more than 50%), limonene, pinene (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40394>, last accessed 2009-11-11). According to Surburg/Panten, the content is limonene (9-16.5%), (-)-carvone (60-70%), menthone (0-0.2%) and viridiflorol (0-0.5%) (34). Exposure by toothpastes, and subsequent contact allergic reaction of the lips or the oral mucosa, have been reported (e.g., (247, 248)). L-Carvone is a component of the oil from *Mentha spicata* (spearment) (53) and had been tested with positive results in “toothpaste cases”, even at a concentration as low as 0.067% (68).

For Oil of spearment -- Part 1: Native type (*Mentha spicata* L.) an ISO standard exists: ISO 3033-1:2005, for Oil of spearment -- Part 2: Chinese type (80 % and 60 %) (*Mentha viridis* L. var. *crispa* Benth.), redistilled oil: ISO 3033-2:2005, for Oil of spearment -- Part 3: Indian type (*Mentha spicata* L.), redistilled oil: ISO 3033-3:2005 and for Oil of spearment -- Part 4: Scotch variety (*Mentha x gracilis* Sole): ISO 3033-4:2005.

MYROXYLON PEREIRAE RESIN

CAS 8007-00-9; EC 232-352-8 (Balsams, Peru)

Balsam of Peru

INCI: MYROXYLON PEREIRAE / Balsams, Peru

Current regulation: Annex III, part1, n° 154

Clinical data:

This natural mixture has been employed as screening agent in Baseline series worldwide for many decades. Hence, a wealth of data is available; table 3.2 – 1 summarises results of the past 10 years.

Additional information:

ISO 4720:2009 nomenclature: *Myroxylon pereirae* (Royle) Klotzsch, syn. *Myroxylon balsamum* var. *pereirae* (Royle) Harms. *Myroxylon pereirae* resin (MPR, Balsamum peruvianum) is harvested from the balsam of Peru tree, *Myroxylon balsamicum* (L.) HARMS var. *pereirae* (ROYLE) HARMS, synonymous *Myroxylon pereirae* (ROYLE) KLOTZSCH (249) after thermal stress, almost exclusively in El Salvador. Main constituents of the pleasantly, vanilla-like smelling dark brown liquid are benzyl esters of cinnamic and benzoic acid (35 – 75%), up to 30% cinnamic acid, up to about 10% benzoic acid, approximately 5% alpha- and beta-nerolidol, benzyl alcohol and mostly less than 1% cinnamyl alcohol, benzyl ferulate and -isoferulate, cinnamic acid amyl ester, coniferyl alcohol, coniferyl benzoate, eugenol, isoeugenol, farnesol, vanillin, and several trace constituents (250-253). The composition of MPR varies with the origin and other factors; moreover, MPR is sometimes blended with other natural mixtures such as turpentine, styrax or colophonium (249).

MPR can be used to improve taste or smell in gargling solutions, cosmetic products such as soaps, shampoo or lipsticks, as well as sweets, tobacco and beverages (249, 254). According to EU legislation and IFRA guidelines MPR should not be used in products intended for skin contact; however, extracts and distillates of MPR may be used in a concentration of < 0,4% (IFRA-Guidelines, www.ifraorg.org (255)). E. Temesvári et al. report on the interesting case of severe ACD with subsequent hypopigmentation after a “temporary henna tattoo”, which was, unexpectedly, not due to p-phenylene diamine, but to the oil used to disperse the pigment, which presumably contained allergens also included in the FM I and MPR, both of which were extreme positive on a later PT (256).

In addition to delayed type hypersensitivity reactions, MPR (and some of his constituents such as benzoic acid (257)) are capable of eliciting (non-immunological) urticarial immediate reactions (258-260). In one case, the immediate reaction to MPR (and to FM I) at the test site spread systemically in terms of a generalised urticaria, while no delayed type reactions were observed to the PT (261). Generally, there is apparently no association of immediate reactions to MPR (and cinnamal or cinnamyl alcohol) and contact sensitisation to these compounds (262). In animal experiments the sensitising potency of MPR was clearly established (250), with coniferyl benzoate identified as single compound with the most marked potency (252). However, due to the limited chemical stability of this compound it is unclear whether other, more stable compounds are, in fact, more important allergens, such as cinnamic acid and (iso-) ferulic acid esters or oxidised constituents of the resin fraction (263).

Table 3.2.2 – 1: Results with contact allergy to fragrance ingredients screening agents reported since 1999 in patients patch tested for suspected allergic contact dermatitis: **Myroxylon pereirae resin** (Balsam of Peru) 1). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the reviewers.

Country	Population	Years	No. tested	Crude % positive (95% CI) §
Tel Aviv, Israel (264) #	Consecutive patients	1999-2000	943	6.6 % (5.1 – 8.4) §
South Korea (13)	Consecutive patients	04/2002 – 06/2003	422	7.3% (5.1 – 10.3%) §

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Tel Aviv, Israel (265)	Consecutive patients	1998-2004	2156	3.6 (2.9 – 4.5) § %
Manipal, India (266)	Dermatitis patients	1989-1998	1780	n=17
Tehran, Iran (267)	Consecutive patients	2002-2004	250	2.4 (0.9 – 5.2) § %
Sevilla, Spain (268)	Consecutive patients	2002-2004	863	5.8 (4.3 – 7.6) § %
Ankara, Turkey (269)	Consecutive patients	1992-2004	1038	2.1 (1.3 – 3.2) § %
Vienna, Austria (22)	Consecutive patients of one clinic	1997-2000	2660	5.4% (4.6 – 6.3%) §
Czech Republic (270)	Consecutive patients	1997-2001	12058	7.3% (6.8 – 7.8) §
Copenhagen, Denmark (271)	Consecutive patients	1985-2007	16173	3.9 (3.6 – 4.2) § %
Sweden (272)	Consecutive patients	2000	3790	6.5%
9 European countries (273) §	Consecutive patients	2002-2003	9672	6.1 %
Germany, 3 Swiss + 1 Austrian Dept. (7)	Consecutive patients	2005-2008	36919	8.0% (7.7 – 8.3%)
10 depts. From 7 EU countries (274) *	Consecutive patients	1996-2000	26210	6.0 %
USA (Canada) (20)	Probably consecutive patients	2003	1603	6.6%
NACDG 2009 (21)	Consecutive patients	2005-2006	4449	11.9%

§ Calculated by reviewers, where possible (if actual numbers were given)

Probably included in (265)

\$ > 5-fold difference between departments

* About 4-fold difference between departments

NARCISSUS SPP. EXTRACT / OIL

CAS: diverse

Narcissus abs.

Current regulation: /

Clinical data:

In the Frosch 2002 b study, 1.3% positive reactions to “narcissus absolute” (2% pet.) were observed in 1606 consecutive (17). The extract used by the PT allergen provider Almirall/Hermal/Trolab has the CAS number 90064-25-8. The IVDK 2010 c study identified 0.5% positive reactions in 2445 consecutively tested patients and 0.6% positive reactions in 809 patients tested in the context of a special series (30).

Additional information:

Commonly used: *Narcissus poeticus* L. According to (30) the maximum observed concentration in Narcissus abs. are (in %): α -terpineol (23.7); trans-Isoeugenol methyl ether (20); benzyl benzoate (20); coumarin (5.7); benzyl alcohol (4.0); Δ^3 -carene (3.4); cinnamyl alcohol (2.5); phenylethyl alcohol (2.2); ethyl palmitate (2.2); phenylpropyl acetate (1.7); 1,8-cineole (1.5); caryophyllene (1.0); benzyl acetate (0.7); isoeugenol (0.5); farnesol (0.3) (also according to (17)) (30).

OCIMUM BASILICUM HERB OIL

CAS 84775-71-3; EC 283-900-8 (*Ocimum basilicum*, ext. = INCI)

Basil Oil (sweet)

Current regulation: /

Clinical data:

/

Additional information:

ISO 4720:2009 nomenclature: *Ocimum basilicum* L. For Oil of basil, methyl chavicol type (*Ocimum basilicum* L.) an ISO standard exists: ISO 11043:1998. *Ocimum Basilicum* Herb Oil is an essential oil obtained from the herbs of the Sweet Basil, *Ocimum basilicum* L., *Labiatae*. (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40474>, last accessed 2009-11-24). The chemical composition varies greatly with the origin (34):

- Basil oil of the methylchavicol type (Réunion type) is extracted from flowering tops or whole plants from Réunion, Comores, Madagascar, but also other countries such as Egypt. Mainly used for seasoning food. Content by GC: methylchavicol 75-87%, linalool 0.5-3%
- Basil oil, linalool type is produced mainly in the Mediterranean area. Content by GC: Linalool 45-62%, methylchavicol trace to 30%, eugenol 2-15%
- Indian Basil oil is produced exclusively in India. Content by GC: methylchavicol trace to 70%, linalool 25%.

In a LLNA study by RIFM, the basil oil as used was reported to contain 51% linalool, 10.4% eugenol, 7.7% cineol, 3.7% bergamotene, 2.7% germacrene D, 2.7% cadinol and 1.3% cadinene, according to analyses of the supplier. The EC3 value was calculated to be < 2.5% (227).

PELARGONIUM GRAVEOLENS FLOWER OIL

CAS 90082-51-2; EC 290-140-0 (*Pelargonium graveolens*, ext. = INCI) / 8000-46-2; EC / (Oils, geranium) INCI: GERANIUM

Geranium Oil Bourbon

Current regulation: /

Clinical data:

The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "geranium oil Bourbon" (2% pet.) 7.4% positive reactions (9). In the Larsen 2001 study, 8.4% positive reactions were observed in 178 patients with known contact allergy to fragrance ingredients ("geranium oil Bourbon", 10% pet.) (19). The Goossens 1997 study found 3 of 111 patients positive to "geranium oil 20% pet." – all sensitised to other fragrance allergens (23). The Rudzki 1976 study found 3 positive reactions in 200 patients to "geranium" essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=2 (2.3%) positive reactions to "geranium" essential oil 2% pet. (27). Nakayama et al. found 1974 (after (29)) 3 "strong positive" reactions to "Geranium oil" (unknown test concentration) in 183 patients, Trattner/David 1 / 641 consecutive patients positive to "Geranium oil" (31). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=8 reacted positively to geranium oil bourbon (48).

Additional information:

ISO 4720:2009 nomenclature: *Pelargonium x ssp.* For Oil of geranium (*Pelargonium X ssp.*) an ISO standard exists: ISO 4731:2006 *Pelargonium Graveolens* Flower Oil is the volatile oil obtained from the flowers of the Bourbon Geranium, *Pelargonium graveolens* L. Hér. Ex Aiton, *P. roseum* Willdenow (and other nondefined hybrids that have developed in different regions of the world) Geraniaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=57527>, last accessed 2009-11-16)(34). The Bourbon type (Réunion, Madagascar) is more valuable than the North African and Chinese products, and differs in characteristic components: (-)-6,9-guaiadiene [36577-33-0] 5-9% in the Bourbon type, and 10-epi-gamma-eudesmol [15051-81-7] 3-6% in the African type, in addition to the main components (-)-citronellol, isomenthone, formates and tiglates. Chinese oil is similar to Bourbon oil, however, it contains more citronellol (32-43%) and lower amounts of linalool (2-4.5%) and geraniol (5-12%) (34).

In a LLNA study by RIFM, the geranium oil as used was reported to contain 41.1% citronellol, 9.8% 2,6-guiadine, 6.2% isomethone, 4.9% geraniol, 2.2% cis-rose oxide, 2.1% linalool, 1.5% geranyl formate, 1.3% phenyl ethyl tiglate, 1.0% trans-rose oxide, and geranyl tiglate and alpha-pinene at < 1%, according to analyses of the supplier. The EC3 value was calculated to be > 50% (227).

PELARGONIUM ROSEUM LEAF OIL

CAS 90082-55-6; EC 290-144-2
(*Pelargonium roseum*, ext. =
INCI)

Geranium Oil; Rose Geranium Oil

Current regulation: /

Clinical data:

In the Sugiura 2000 study, among 1483 patients with suspected cosmetic dermatitis, 2.1% positive PT reactions to "geranium oil" (tested 20% in pet.) were observed (14).

Additional information:

Pelargonium Roseum Leaf Oil is an essential oil obtained from the leaves of the plant, Pelargonium roseum, Geraniaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40565>, last accessed 2009-11-16).

PIMENTA RACEMOSA LEAF/FRUIT OIL

CAS 85085-61-6; EC 285-385-5

Bay oil (34)

Current regulation: /

Clinical data:
/

Additional information:

ISO 4720:2009 nomenclature: *Pimenta racemosa* (Mill.) J.W. Moore. For Oil of bay [*Pimenta racemosa* (Mill.) J.W. Moore] an ISO standard exists: ISO 3045:2004 Pimenta Racemosa Leaf/Fruit Oil is an essential oil obtained from the fruits of the plant, Pimenta racemosa, Myrtaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41014>, last accessed 2010-02-10).

Steam distillation of the leaves of Pimenta racemosa (Mill.) J.W. Moore (Myrtaceae) yields bay oil, which consists of myrcene (20-30%), eugenol (42-56%) and chavicol (8-13%) (34).

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

Pinus mugo leaf and twig oil and extract

CAS 90082-72-7, 8000-26-8; EC 290-163-6

Dwarf pine needle oil
(German: Latschenkiefernöl)

Current regulation: Annex III, part 1, 109

Clinical data:

In the Frosch 2002 b study, 0.7% positive reactions to dwarf pine needle oil (2% pet.) were observed in 1606 consecutive (17). The Rudzki 1976 study found 4 positive reactions in 200 patients to "Pine needle" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to "pine needle" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Pinus mugo* Turra syn. *Pinus montana* Mill.) Pinus Mugo Twig Oil is an essential obtained from the twigs of the Pine, Pinus mugo, Pinaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.h.details&id=41476&back=1>, last accessed 2010-03-09). Pinus Mugo Twig Leaf Extract is an extract obtained from the twigs leaves of the Pine, Pinus mugo, Pinaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.h.details&id=41476&back=1>, last accessed 2010-03-09).

[h.details&id=41473&back=1](#), last accessed 2010-03-09).

Dwarf pine needle oil is obtained from *Pinus mugo* Turra subsp. *mugo* and subsp. *pumilio* (Haenke) Franco (34). For Oil of dwarf pine (*Pinus mugo* Turra) an ISO standard exists: ISO 21093:2003. American pine oils contain almost no 3-carene or camphene (34).

PINUS PUMILA TWIG LEAF EXTRACT / OIL

CAS 97676-05-6; EC 307-681-6
(Pine, *Pinus pumila*, ext. = INCI)

Dwarf pine needle oil

Current regulation: Annex III, part 1, 114

Clinical data: /

Additional information:

Pinus Pumila Twig Leaf Extract obtained from the twigs leaves of the Pine, *Pinus pumila*, Pinaceae

(<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41483&back=1>, last accessed 2009-11-12), *Pinus Pumila* Twig Leaf Oil is the essential oil obtained from the twigs leaves of the Pine, *Pinus pumila*, Pinaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41484&back=1>, last accessed 2009-11-12). Main constituents are alpha-pinene (60-70%) and beta-pinene (20-25%). (34) Occurrence from Siberia to Japan, classified as Endangered Species

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

POGOSTEMON CABLIN OIL

CAS 8014-09-3; EC / (Oils, patchouli) / 84238-39-1; EC 282-493-4 (Patchouli, ext.)

Patchouli oil

INCI: POGOSTEMON CABLIN / Patchouli, ext.

Current regulation: /

Clinical data:

In the Frosch 2002 b study, 0.8% positive reactions to patchouli oil (10% pet.) in 1606 consecutive were observed (17). Nakayama et al. found 1974 (after (29)) 3 “strong positive” and 8 “weak positive” reactions to “Patchouli oil” (unknown test concentration) in 183 patients. The IVDK 2010 c study identified 0.6% positive reactions in 2446 consecutively tested patients and 1.4% positive reactions in 828 patients tested in the context of a special series (30).

Additional information:

ISO 4720:2009 nomenclature: *Pogostemon cablin* (Blanco) Benth. syn. *Mentha cablin* Blanco. An ISO standard is available for Oil of patchouli (*Pogostemon cablin* (Blanco) Benth.): ISO 3757:2002. *Pogostemon Cablin* Leaf Oil is an essential oil obtained from the fermented leaves of the Patchouli, *Pogostemon cablin* (syn: *Pogostemon patchouli*),

Labiatae (Lamiaceae (34)). It contains patchouli alcohol, beta-patchoulene, azulene, eugenol, sesquiterpenes (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40927>, last accessed 2009-11-12). Although the sesquiterpene alcohol (-)-patchoulol [5986-55-0] is the main component of patchouli oil (27-35%), the compound largely contributing to the characteristic odour is norpatchoulol [41429-52-1] (0.35-1%). Other constituents include (+)-alpha-bulnesene [6391-11-0] (13-21%), (-)-alpha-guajene [3691-12-1] (11-16%), (-)-β-patchoulene [514-51-2] (1.8-3.5%) and (-)-seychellene [20085-93-2] (1-3%) (34). According to (30) the maximum observed concentration in patchouli oil are (in %): (-)-patchoulol (35); (+)-alpha-lulnesene (21); (-)-alpha-guajene (16); β-pinene (6); (-)-β-patchoulene (3.5); (-)-seychellene (3); pogostol (2.5); α-pinene (2.5); norpatchoulol (1) (30).

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

ROSE FLOWER OIL (ROSA SPP.)

CAS 8007-01-0; EC / (Oils, rose)

ROSA ALBA FLOWER EXTRACT

CAS 93334-48-6; EC 297-122-1
(Rose, *Rosa alba*, ext. = INCI)

ROSA CANINA FLOWER OIL

CAS 84696-47-9; EC 283-652-0
(Rose, *Rosa canina*, ext.) INCI:
ROSA CANINA

ROSA CENTIFOLIA FLOWER OIL

CAS 84604-12-6, EC 283-289-8
(Rose, *Rosa centifolia*, ext.) INCI:
ROSA CENTIFOLIA / Rose, *Rosa centifolia*, ext.

ROSA DAMASCENA FLOWER OIL

CAS 90106-38-0; EC 290-260-3
(Rose, *Rosa Damascena*, ext. = INCI)

ROSA GALLICA FLOWER OIL

CAS 84604-13-7; EC 283-290-3
(Rose, *Rosa Gallica*, ext.) INCI:
ROSA GALLICA

ROSA MOSCHATA OIL

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ROSA RUGOSA FLOWER OIL

CAS 92347-25-6; EC 296-213-3
(Rose, *Rosa rugosa*, ext.)

Current regulation: /

Clinical data:

In the Sugiura 2000 study, 1483 patients with suspected cosmetic dermatitis were PTed with "rose oil Bulgaria" (2% pet.), yielding 0.4% positive reactions (14); Trattner/David found 2 / 641 consecutive patients positive to "Rose oil (Bulgarian)" (31). The Bulgarian rose oil usually corresponds to *Rosa Damascena* Flower Oil (http://en.wikipedia.org/wiki/Rose_oil, last accessed 2009-11-16). The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "rose Bulgarian oil" (2% pet.) 4.5% positive reactions (9). One case of contact allergy to "Bulgarian rose oil (2 % pet.)" – and geraniol – in a 48-year-old female with ACD after application of "Eau de Rochas" E.d.C. was diagnosed, among 326 patients with suspected contact allergy to fragrance ingredients had tested negative (275). However, other rose oils are also used (and capable of eliciting ACD) as illustrated by the case of a 27 year old woman who developed ACD after using "Rose Absolute Eau ® eau de

parfum", a "non-scented" body lotion and a number of other topicals. PTing revealed a number of (previously) relevant reaction, including "Rose centifolia" (5% alc.) and "Rose oil Bulgarian" (2% pet.) essential oil preparations (276). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had positive reactions to "Rose oil Bulgarian", tested at 2% concentration (13). Nakayama et al. found 1974 (after (29)) 4 "strong positive" reactions to "Rose oil Bulgarian" (unknown test concentration) in 183 patients. In a study from Alicante, Spain, 86 selected patients were tested with rose oil absolute, yielding 6 positive reactions (48).

Additional information:

ISO 4720:2009 nomenclature: *Rosa x damascena* Mill. and *Rosa sertata* X *Rosa rugosa*. Rose Flower Oil is the volatile oil obtained from the flowers of *Rosa* spp. , rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=59362>, last accessed 2009-11-16). "Rose oil, meaning either rose otto (attar of rose, attar of roses) or rose absolute, is the essential oil extracted from the petals of various types of rose. Rose ottos are extracted through steam distillation, while rose absolutes are obtained through solvent extraction or supercritical carbon dioxide extraction, with the absolute being used more commonly in perfumery" (http://en.wikipedia.org/wiki/Rose_oil, last accessed 2009-11-17). There are several more specifically named flower extracts used for masking or perfuming:

- Rosa Alba Flower Extract is an extract obtained from the flowers of the Rose, *Rosa alba* L., Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40969>, last accessed 2009-11-16).
- Rosa Canina Flower Oil is the volatile oil obtained from the flowers of the Hip Rose, *Rosa canina* L., Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=59263>, last accessed 2009-11-16).
- Rosa Centifolia Flower Oil is the volatile oil obtained from the flowers of the Cabbage Rose, *Rosa centifolia* (L.), Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=79757>, last accessed 2009-11-16).
- Rosa Damascena Flower Oil is the volatile oil obtained from the flowers of the Damask Rose, *Rosa damascena*, Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=79760>, last accessed 2009-11-16).
- Rosa Gallica Flower Oil is the volatile oil obtained from the flowers of the French Rose, *Rosa gallica* L., Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=59346>, last accessed 2009-11-16).
- Rosa Moschata Oil is the oil obtained from the Musk Rose, *Rosa moschata*, Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=79761>, last accessed 2009-11-16).
- Rosa Rugosa Flower Oil is the volatile oil obtained from the flowers of the Rose, *Rosa rubiginosa* L., Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=83588>, last accessed 2009-11-16).

Apparently, the *Rosa Damascena* and the *Rosa centifolia* are the species most commonly used for extraction of essential rose oils, the former mostly grown in Bulgaria, Turkey, Russia, India and China, the latter more commonly in Morocco, France and Egypt (276). Main constituents by GC are: citronellol (20-49%), geraniol (6-23%), nerol (3-12%) and phenylethyl alcohol (up to 3.5%) (34).

For Oil of rose (*Rosa x damascena* Miller) an ISO standard exists: ISO 9842:2003.

ROSMARINUS OFFICINALIS FLOWER OILCAS 84604-14-8; EC 283-291-9
(Rosemary, ext.)

"Rosemary Oil"

INCI: ROSMARINUM OFFICINALIS
/ Rosemary, ext.

Current regulation: /

Clinical data:

The Rudzki 1976 study found no positive reaction in 200 patients to "rosemary" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to "rosemary" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Rosmarinus officinalis* L. Rosmarinus Officinalis Flower Oil is an essential oil obtained from the leaves and fresh flowering tops of the Rosemary, *Rosmarinus officinalis* L., Lamiaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40978>, last accessed 2010-01-29). Major constituents are: 1,8-cineole (17-55%), alpha-pinene (9-26%), camphor (5-22%) and verbenone [18309-32-5] as traces in North African oils, but between 0.7 and 2.5% in Spanish oils (34). For Oil of rosemary (*Rosmarinus officinalis* L.) an ISO standard exists: ISO 1342:2000.

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

SALVIA spp. HERB OIL

Sage oil

SALVIA OFFICINALIS LAVANDULIFOLIA HERB OILCAS 97952-71-1; EC 308-365-0
(Sage, *Salvia officinalis* *lavandulifolia*, ext. = INCI)**SALVIA LAVANDULIFOLIA HERB OIL**CAS 90106-49-3; EC 290-272-9
(Sage, *Salvia lavandulifolia*, ext. = INCI)**SALVIA SCLAREA FLOWER OIL**CAS 84775-83-7; EC 283-911-8
(Sage, *Salvia sclarea*, ext.) INCI: SALVIA SCLAREA / Sage, *Salvia sclarea*, ext.**SALVIA HISPANICA HERB OIL**CAS 93384-40-8; EC 297-250-8
(Sage, *Salvia hispanica*, ext. = INCI)

Current regulation: /

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to "Clary sage", 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=4 (4.6%) positive reactions to "clary sage" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Salvia officinalis* L. *Salvia Officinalis Lavandulifolia* Herb Oil is an essential oil obtained from the herbs of the Sage, *Salvia officinalis* L. spp. *lavandulifolia*, Lamiaceae, Syn. Dalmatian sage (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41084>, last accessed 2010-01-29).

Salvia Lavandulifolia Herb Oil is an essential oil obtained from the herbs of the Sage, *Salvia lavandulifolia*, Lamiaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40987>, last accessed 2010-01-29).

Salvia Sclarea Flower Oil is an essential oil obtained from the flowers and foliage of the Clary Sage, *Salvia sclarea* L., Lamiaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41086>, last accessed 2010-01-29).

Salvia Hispanica Herb Oil is an essential oil obtained from the herbs of the Spanish Sage, *Salvia hispanica* L., Lamiaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40985>, last accessed 2010-01-29).

Clary sage oil is obtained by steam distillation of flowering tops and foliage of cultivated *Salvia sclarea* L. (Lamiaceae). Main constituents are linalyl acetate (56-78%) and linalool (6.5-24%) (34). Dalmatian sage oil is steam distilled from partially dried leaves of *S. officinalis* L. (Lamiaceae). The content by GC is: alpha-thujone (18-43%), beta-thujone (3-8.5%), 1,8-cineole (5.5-13%), camphor (3-8.5%) as main constituents (34). Spanish sage oil does not contain thujone, but mainly camphor (15-36%) and 1,8-cineole (11-30%), and is used mainly in pharmaceutical preparations and technical perfumery (34). For Oil of sage, Spanish (*Salvia lavandulifolia* Vahl) an ISO standard exists: ISO 3526:2005, for Oil of Dalmatian sage (*Salvia officinalis* L.): ISO 9909:1997.

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

SANTALUM ALBUM WOOD OIL

CAS 84787-70-2; EC 284-111-1
(Sandalwood, ext.) INCI:
SANTALUM ALBUM / Sandalwood,
ext.

Sandalwood oil ([East] India)

SANTALUM ALBUM OIL

CAS 8006-87-9; EC / (Oils,
sandalwood)

Sandalwood oil ([East] India)

Current regulation: /

Clinical data:

In the Sugiura 2000 study, 1483 patients with suspected cosmetic dermatitis were PTed with "sandalwood oil" (2% pet.), yielding 0.8% positive reactions (14). In the Frosch 2002 b study, "sandalwood oil (East India)" is mentioned with a CAS # 8015-65-4, which, however, is attributed to AMYRIS BALSAMIFERA BARK OIL, see above. Assuming that this CAS # is erroneous, study results are considered to be valid for *S. album* wood oil, tested at 2% and 10% concentration, yielding 0.4% and 0.9% positive reactions,

respectively (17). Out of 6 of 15 patients with a positive reaction to the higher concentration no clinical relevance was found, compared to 2 of 7 patients positive to the lower concentration (17). The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "sandalwood oil" (2% pet.) 6.6% positive reactions (9). In the An 2005 study, 10 of 422 consecutive patients, i.e., 2.4%, had positive reactions to "Santalum album oil" 2% (13). The Goossens 1997 study found 4 of 111 patients positive to "sandalwood oil 10% pet." – all sensitised to other fragrance allergens (23). The Rudzki 1976 study found no positive reaction in 200 patients to "sandalwood", 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=2 (2.3%) positive reactions to "sandalwood" essential oil 2% pet. (27). In 63 patients positive to the FM I, 1 had a positive PT reaction to sandalwood oil, 2% pet., in the Santucci 1987 study (28). Nakayama et al. found 1974 (after (29)) 6 "strong positive" and 8 "weak positive" reactions to "Sandalwood oil" (unknown test concentration) in 183 patients. The IVDK 2010 c study identified 1.3% positive reactions in 3671 consecutively tested patients and 1.8% positive reactions in 1002 patients tested in the context of a special series (30). In a study from Alicante, Spain, 86 selected patients were tested with sandalwood oil, yielding 2 positive reactions (48).

Additional information:

ISO 4720:2009 nomenclature: *Santalum album* L. *Santalum Album* Oil is the volatile oil obtained from the heartwood of the Sandalwood, *Santalum album* L., Santalaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=80209>, last accessed 2009-11-26).

Santalum Album Wood Oil is an essential oil obtained from the wood of the Sandalwood, *Santalum album* L., Santalaceae. It contains 75% santalol isomers (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41092>, last accessed 2009-11-12), typically up to 55% .alpha.-santalol and up to 24% .beta.-santalol (30). East Indian sandalwood oil consists almost exclusively of closely related sesquiterpenoids; by far the main constituents are the alcohols alpha-santalol [115-71-9] (41-55%) and cis-beta-santalol [77-42-9] (16-24%), the latter being mainly responsible for the specific odour (34, 39).

An ISO standard regarding the composition of "*Santalum album* oil" is available: ISO 3518:2002. "Sandalwoods" are labelled as *Amyris balsamifera*, *Eremophila mitchelli*, *Fusanus acuminatus* (= *Santalum acuminatum*), *Santalum album*, *S. austrocaledonicum*, *S. latifolium*, *S. spicatum* and *S. yasi*. The majority of currently available trade oils, reportedly from *S. album*, contained approximately 50-70% santalols (Z-alpha and Z-beta), as analysed with gas chromatography-mass spectrometry (GC-MS) (277). A review on the toxicological properties of "*Santalum album* oil" is available (278).

AMYRIS BALSAMIFERA BARK OIL (*Sandalwood oil (Caribbean)*), CAS 8015-65-4; EC / (Oils, amyris) / 90320-49-3; EC 90320-49-3 (*Amyris balsamifera*, ext. = INCI name) is used as a cheap substitute for East Indian Sandalwood in perfumes and cosmetics. Originally cultivated primarily in Haiti where it was known as 'candle wood' and used as a torch by locals due to the tree's high oil content (<http://www.amphora-retail.com/sandalwood-amyris-essential-10ml-p-107.html>, last accessed 2009-11-12). The major components are sesquiterpenoids such as valerianol, elemol, β -eudesmol and epi-gamma-eudesmol (39). For Oil of amyris (*Amyris balsamifera* L.) an ISO standard exists: ISO 3525:2008. *Amyris Balsamifera* Bark Oil is the volatile oil distilled from the bark of the tree, *Amyris balsamifera*, Rutaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=74455>, last accessed 2009-11-12).

SANTALUM SPICATA WOOD OILCAS 8024-35-9; EC 296-618-5
(Sandalwood oil, Western
Australia)*Sandalwood oil (Australia)*

Current regulation: /

Clinical data:

In clinical studies, mostly *S. album* wood oil had been used (see above); in a number of studies this is not clear.

Additional information:

ISO 4720:2009 nomenclature: *Santalum spicatum* (R.Br.) A. DC, syn. *Eucarya spicata* (R.Br.) Sprag & Summ. For Oil of Australian sandalwood (*Santalum spicatum* (R.Br.) A.DC.) an ISO standard exists: ISO 22769:2009. Santalum Spicata Wood Oil is an essential oil obtained from the wood of the Australian Sandalwood, *Santalum spicata*, Santalaceae. It contains 75% santalols and 10% farnesol (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41093>, last accessed 2009-11-12). This oil also contains santalols as main constituents but differs somewhat in the remaining composition. Today, it makes up a considerable part of the sandalwood oil market (34).

Considering the content of well-known allergenic compounds (santalols), this essential oil is regarded as established contact allergen in humans.

TAGETES PATULA FLOWER OILCAS 91722-29-1; EC 294-431-3
(*Tagetes patula*, ext. = INCI)*"Marigold Oil; Tagetes Oil"*

Current regulation: /

Clinical data:

In an aromatherapist, an essential oil solvent-extracted from *Tagetes patula*, patch tested at 1.5% in grapeseed oil (vehicle negative, 7 controls negative to essential oils) resulted in a +++ reaction, in accordance with a work-related bilateral hand dermatitis (217).

Additional information:

Tagetes Patula Flower Oil is an essential oil obtained by hydrodistillation of the flowers of the *Tagetes*, *Tagetes patula* L., *Compositae*. It contains mainly D-limonene, ocimene, 2,6-dimethyloct-7-en-4-one (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41506>, last accessed 2010-01-28). According to Surburg/Panten, tagetes oil is steam distilled from the flowering plants of *Tagetes minuta* L. (*T. glandulifera* Schrank., *Asteraceae*). Main components comprise cis-ocimene, dihydrotagetone, tagetone, and cis- and trans-ocimenone (34, 39).

THYMUS spp. HERB OIL

THYMUS VULGARIS HERB OIL

CAS 84929-51-1, 8007-46-3; EC 284-535-7 (Thyme, Thymus vulgaris, ext.)

"Thyme oil"

INCI: THYMUS VULGARIS / Thyme, Thymus vulgaris, ext.

Current regulation: /

Clinical data:

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

Additional information:

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

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

TURPENTINE (oil)

CAS 8006-64-2 / 9005-90-7 / 8052-14-0; EC 232-350-7 / 232-688-5 / -

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

Clinical data:

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

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

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

Country	Population	Years	No. tested	Crude % positive (95% CI) §

Opinion on fragrance allergens in cosmetic products

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

Additional information:

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

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

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

Verbena absolute (Lippia citriodora Kunth.)

CAS 8024-12-2, 84961-67-1; EC /)

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

Clinical data: /

Additional information:

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

VETIVERIA ZIZANOIDES ROOT OIL

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

"Vetiver oil; khas khas oil"

Current regulation: ...

Clinical data:

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

Additional information:

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

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Annex II - Animal Data

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

Substance		Vehicle (AOO=acetone:olive oil; DEP=diethyl phthalate; DMF=dimethyl formamide; DMSO=dimethyl sulphoxide; EtOH=ethanol; MEK=methyl ethyl ketone)	Conc. in vehicle (%, generally w/v)	No. animals per dose group	EC3 value *				Comment (deviation from OECD 429 etc)	Reference
INCI name (other name)	CAS no.				%	µg/cm ²	M	lowest for the substance (%)		
<i>Allyl phenoxyacetate</i>	7493-74-5	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	4	3.1	775	0.16	3.1		RIFM, 2007a
Amyl cinnamal	122-40-7	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	7.6	1900	0.38	7.6		RIFM, 2006a
Amyl cinnamal	122-40-7	4:1 AOO	-	4	10.6	2650	0.52		Elahi gives ref to Basketter et al 1999, but no data on the substance is found. It is not known if Elahi, Aptula and Roberts quote the same experiment	Elahi et al., 2004

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

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

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

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

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

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

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

EtOH:DEP

Cinnamal	104-55-2	0.1% Trolox C in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	0.7	175	0.053		RIFM, 2003e
Cinnamal	104-55-2	2.0% α -tocopherol in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	0.8	200	0.060		RIFM, 2003f
Cinnamal	104-55-2	3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	0.9	225	0.068		RIFM, 2003g
Cinnamal	104-55-2	0.1% α -tocopherol in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	1.1	275	0.083		RIFM, 2003h
Cinnamal	104-55-2	0.3% antioxidant mix (equal parts BHT, tocopherol and eugenol) in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	1.3	325	0.098		RIFM, 2003i
Cinnamal	104-55-2	0.1% Trolox C in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	1.4	350	0.11		RIFM, 2003j
Cinnamal	104-55-2	-	-	-	-	-	-	No data in the ref (poster abstract)	Basketter et al., 2002
Cinnamal	104-55-2	4:1 AOO	0.5, 1, 2.5, 5, 10	4	3.1	775	0.23		Basketter et al., 2001
Cinnamal	104-55-2	4:1 AOO	-	4	1.3	325	0.10		Elahi et al., 2004
Cinnamal	104-55-2	4:1 AOO	1, 2.5	-	1.4	348	0.11	Too few concentrations tested; few details given in ref	Smith and Hotchkiss, 2001
Cinnamal	104-55-2	4:1 AOO	1.0, 2.5, 5.0, 10.0, 25.0	4	1.7	425	0.13		Wright et al., 1995

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

Cinnamal	104-55-2	MEK	1.0, 2.5, 5.0, 10.0, 25.0	4	1.1	275	0.083		Wright et al., 1996
Cinnamal	104-55-2	DMF	0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0	4	0.5	125	0.038		Wright et al., 1997
Cinnamal	104-55-2	propylene glycol	1.0, 2.5, 5.0, 10.0, 25.0	4	1.4	350	0.11		Wright et al., 1998
Cinnamal	104-55-2	DMSO	0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0	4	0.9	225	0.068		Wright et al., 1999
Cinnamal	104-55-2	90:10 EtOH:water	1.0, 2.5, 5.0, 10.0, 25.0	4	1.6	400	0.12		Wright et al., 2000
Cinnamal	104-55-2	50:50 EtOH:water	1.0, 2.5, 5.0, 10.0, 25.0	4	1.2	300	0.091		Wright et al., 2001
Cinnamyl alcohol	104-54-1	-	-	-	-	-	-	No data in the ref (poster abstract)	Basketter et al., 2002
Cinnamyl nitrile	1885-38-7	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	> 10	>2500	>0.77	> 10	Report: systemic toxicity at 25% and 50%. Should have been tested at higher concentrations RIFM, 2005f
Citral	5392-40-5	1:3 EtOH:DEP	0.4, 2.0, 4.0, 8.0, 20.0	4	1.2	300	0.079	1.2	RIFM, 2004b
Citral	5392-40-5	0.1% α -tocopherol in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	1.5	375	0.099		RIFM, 2003k

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

Citral	5392-40-5	0.3% antioxidant mix (equal parts BHT, tocopherol and eugenol) in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	2.1	525	0.14		RIFM, 2003l
Citral	5392-40-5	0.1% Trolox C in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	3.7	925	0.24		RIFM, 2003m
Citral	5392-40-5	3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	4.6	1150	0.30		RIFM, 2003n
Citral	5392-40-5	0.3% antioxidant mix (equal parts BHT, tocopherol and eugenol) in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	4.6	1150	0.30		RIFM, 2003o
Citral	5392-40-5	3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	5.3	1325	0.35		RIFM, 2003p
Citral	5392-40-5	0.1% Trolox C in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	5.8	1400	0.38		RIFM, 2003q
Citral	5392-40-5	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	6.3	1575	0.41		RIFM, 2003r
Citral	5392-40-5	0.1% α -tocopherol in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	6.8	1700	0.44		RIFM, 2003s
Citral	5392-40-5	-	-	-	-	-	-	No data in the ref (poster abstract)	Basketter et al., 2002
Citronellol	106-22-9	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	43.5	10875	2.78	43.5	RIFM, 2004c

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

Coumarin	91-64-5	DMF	10, 25, 50	4	>50	>12500	>3.42	>50	Should have been tested at higher concentrations	Vocanson et al., 2006
<i>Dibenzyl ether</i>	103-50-4	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	6.3	1575	0.32	6.3		RIFM, 2007e
Eugenol	97-53-0	3:1 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	5.3	1325	0.32	5.3		RIFM, 2001f
Eugenol	97-53-0	1:3 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	10.5	2625	0.64			RIFM, 2001g
Eugenol	97-53-0	EtOH	1.0, 3.0, 10.0, 30.0, 50.0	4	10.7	2675	0.65			RIFM, 2001h
Eugenol	97-53-0	DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	15.1	3775	0.92			RIFM, 2001i
Eugenol	97-53-0	4:1 AOO	2.5, 5.0, 10.0, 25.0, 50.0	-	11.9	2975	0.72			Basketter et al., 1999
Eugenol	97-53-0	-	-	-	-	-	-		No data in the ref (poster abstract)	Basketter et al., 2003
Evernia furfuracea extract <i>Treemoss absolute</i>	90028-67-4	1:3 EtOH:DEP	5.0, 10.0, 20	4	> 20	>5000	N/a	> 20	Should have been tested at higher concentrations	RIFM, 2004k
Evernia furfuracea extract <i>Treemoss absolute</i>	90028-67-4	1:3 EtOH:DEP	10.0, 25.0	4	> 25	>6250	N/a		Too few concentrations tested	RIFM, 2004d

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Evernia prunastri extract <i>Oakmoss</i>	90028-68-5	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	3.88	970	N/a	3.88		RIFM, 2004j
Farnesol	4602-84-0	4:1 AOO	5.0, 10.0, 25.0	4	5.5	1375	0.25		Should also have been tested at lower concentrations	RIFM, 2004d
Farnesol	4602-84-0	4:1 AOO	5.0, 10.0, 25.0	4	4.1	1025	0.18	4.1	Should also have been tested at lower concentrations	RIFM, 2004d
Geraniol	106-24-1	EtOH	1.0, 3.0, 10.0, 30.0, 50.0	4	5.6	1400	0.36	5.6		RIFM, 2001j
Geraniol	106-24-1	3:1 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	11.4	2850	0.74			RIFM, 2003t
Geraniol	106-24-1	DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	11.8	2950	0.76			RIFM, 2001k
Geraniol	106-24-1	1:3 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	20.4	5100	1.32			RIFM, 2001l
Geraniol	106-24-1	3:1 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	25.8	6450	1.67			RIFM, 2001m
Geraniol	106-24-1	-	-	-	26	6500	1.69			Roberts et al., 2007
<i>trans-2-Hexenal</i>	6728-26-3	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5, 10	4	2.6	650	0.26	2.6		RIFM, 2005g
<i>trans-2-Hexenal</i>	6728-26-3	-	-	-	5.5	1375	0.56			Roberts et al., 2007

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

Hexyl cinnamal	101-86-0	generally 4:1 AOO	-O162	5.3-14.7	1325-3675	0.25-0.68	5.3		"numerous accounts in the literature"
2-Hexylidene cyclopentanone	17373-89-6	1:3 EtOH:DEP	0.1, 0.5, 1.0, 2.5, 5.0	5	2.4	600	0.14	2.4	RIFM, 2008a
Hexyl salicylate	6259-76-3	1:3 EtOH:DEP	0.05, 0.25, 0.5, 1.0, 2.5	4	0.18	45	0.008	0.18	RIFM, 2006b
Hydroxycitronellal	107-75-5	1:3 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	19.3	4825	1.12	19.3	RIFM, 2001n
Hydroxycitronellal	107-75-5	DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	19.7	4925	1.14		RIFM, 2001o
Hydroxycitronellal	107-75-5	3:1 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	22.2	5550	1.29		RIFM, 2001p
Hydroxycitronellal	107-75-5	EtOH	1.0, 3.0, 10.0, 30.0, 50.0	4	26.4	6600	1.53		RIFM, 2001q
Hydroxycitronellal	107-75-5	AOO	25, 50, 100	-	-	-	-	EC3 value not given	Ashby et al., 1995
Hydroxycitronellal	107-75-5	4:1 AOO	2.5, 5, 10, 25, 50	4	33.0	8250	1.92		Basketter et al., 2001
Hydroxycitronellal	107-75-5	-	-	-	-	-	-	No data in the ref (poster abstract)	Basketter et al., 2002
Hydroxycitronellal	107-75-5	-	-	-	25.25	6313	1.47		Estrada et al., 2003

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Hydroxycitronellal	107-75-5	4:1 AOO	10, 25	-	23	5750	1.34		Too few concentrations tested; few details given in ref	Smith and Hotchkiss, 2001
Hydroxyisohexyl 3-cyclohexene carboxaldehyde	31906-04-4	4:1 AOO	1.0, 2.5, 5, 10, 25, 50	4	17.1	4275	0.81	17.1		RIFM, 2001r
<i>p</i> -Isobutyl- α -methyl hydrocinnamaldehyde	6658-48-6	70% EtOH	10.0, 25.0, 50.0, 100.0	4	9.5	2375	0.46	9.5	Should also have been tested at lower concentrations	RIFM, 2001w
<i>Isocyclocitral</i>	1335-66-6	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	4	7.3	1825	0.48	7.3		RIFM, 2006c
<i>Isocyclogeraniol</i>	68527-77-5	1:3 EtOH:DEP	5.0, 10.0, 25.0, 50.0	4	> 25	>6250	>1.62	> 25	Should have been tested at higher concentrations	RIFM, 2005h
Isoeugenol	97-54-1	4:1 AOO	0.5, 5.0	6	0.54	145	0.033	0.54	Too few concentrations tested	RIFM, 2001s
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	0.6	150	0.037			RIFM, 2002a
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	0.76	191	0.046			RIFM, 2002b
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	0.79	199	0.048			RIFM, 2002c

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Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	1.19	296	0.072	RIFM, 2001t
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	1.28	320	0.078	RIFM, 2004e
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	6	1.54	385	0.094	RIFM, 2001u
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	1.95	488	0.119	RIFM, 2001v
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0		3.3	825	0.20	Basketter et al., 1999
Isoeugenol	97-54-1	-	-	-	-	-	-	No data in the ref (poster abstract) Basketter et al., 2002
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	1.3	325	0.079	Loveless et al., 1996
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	3.3	825	0.20	Loveless et al., 1996
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	1.8	450	0.11	Loveless et al., 1996
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	3.1	775	0.19	Loveless et al., 1996
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	1.6	400	0.097	Loveless et al., 1996
Isoeugenol	97-54-1	AOO	0.5, 1.0, 2.5, 5.0, 10.0	4	1.0	250	0.061	Wright et al., 2001
Isoeugenol	97-54-1	MEK	0.5, 1.0, 2.5, 5.0, 10.0	4	1.0	250	0.061	Wright et al., 2001
Isoeugenol	97-54-1	DMF	0.5, 1.0, 2.5, 5.0, 10.0	4	1.4	350	0.085	Wright et al., 2001

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

Isoeugenol	97-54-1	propylene glycol	0.5, 1.0, 2.5, 5.0, 10.0	4	2.5	625	0.15			Wright et al., 2001
Isoeugenol	97-54-1	DMSO	0.5, 1.0, 2.5, 5.0, 10.0	4	0.9	225	0.055			Wright et al., 2001
Isoeugenol	97-54-1	90:10 EtOH:water	0.5, 1.0, 2.5, 5.0, 10.0	4	1.8	450	0.11			Wright et al., 2001
Isoeugenol	97-54-1	50:50 EtOH:water	0.5, 1.0, 2.5, 5.0, 10.0	4	4.9	1225	0.30			Wright et al., 2001
<i>Jasmine absolute (Grandiflorum)</i>	8022-96-6	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	5.9	1475	N/a	5.9		RIFM, 2006d
Jasminum Sambac Flower CERA / Extract / Water	91770-14-8	1:3 EtOH:DEP	10.0, 25.0, 50.0, 75.0, 100.0	4	35.4	9100	N/a	35.4		RIFM, 2006e
<i>d-Limonene**</i>	5989-27-5	EtOH	10.0, 20.0, 50.0, 75.0, 100.0	4	< 10	< 250	<0.73	< 10	Should also have been tested at lower concentrations	RIFM, 2004l
<i>d-Limonene**</i>	5989-27-5	3:1 EtOH:DEP	10.0, 20.0, 50.0, 75.0, 100.0	4	22.0	5500	1.61			RIFM, 2004m
<i>d-Limonene**</i>	5989-27-5	1:3 EtOH:DEP	10.0, 20.0, 50.0, 75.0, 100.0	4	38.0	9500	2.79			RIFM, 2004n
<i>d-Limonene**</i>	5989-27-5	DEP	10.0, 20.0, 50.0, 75.0, 100.0	4	63.0	15.75	4.62			RIFM, 2004o
<i>d-Limonene**</i>	5989-27-5	4:1 AOO	25, 50, 100	4	68.5	17125	5.03			Warbrick et al., 2001

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

Linalool**	78-70-6	-	-	-	-	-	-	-	No data in the ref (poster abstract)	Basketter et al., 2002
Menthadiene-7-methyl formate	68683- 20-5	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	5	> 10	> 2500	>0.51	> 10	Should have been tested at higher concentrations	RIFM, 2008c
4-Methoxy- α -methyl benzenpropanal	5462- 06-6	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	5	23.6	5900	1.32	23.63		RIFM, 2004f
α -Methyl cinnamic aldehyde	101-39- 3	-	-	-	4.5	1125	0.31	4.5		Roberts et al., 2007
Methylenedioxyphenyl methylpropanal	1205- 17-0	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	16.4	4100	0.85	16.4		RIFM, 2005i
6-Methyl-3,5-heptadien-2- one	1604- 28-0	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	5	> 5	> 1250	>0.40	> 5	Should have been tested at higher concentrations	RIFM, 2008d
α -iso-Methylionone	127-51- 5	1:3 EtOH:DEP	10.0, 25.0, 50.0, 75.0, 100.0	4	21.8	5450	1.06	21.8		RIFM, 2005j
Methyl octine carbonate	111-80- 8	-	-	-	2.5	635	0.15	2.5		Roberts et al., 2007

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Methyl 2-octynoate	111-12-6	1:3 EtOH:DEP	0.5, 1.0, 2.0, 5.0, 10.0	4	< 0.5	< 125	<0.032	< 0.5	Should also have been tested at lower concentrations	RIFM, 2005k
2-Methoxy-4-methylphenol	93-51-6	-	-	-	5.8	1450	0.42	5.8		Roberts et al., 2007
1-Octen-3-yl acetate	2442-10-6	1:3 EtOH:DEP	7.5, 15.0, 30.0	5	> 30	> 7500	>1.76	> 30	Should have been tested at higher concentrations	RIFM, 2004g
Perillaldehyde <i>p</i> -Mentha-1,8-dien-7-al	2111-75-3	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	5	9.3	2325	0.62			RIFM, 2008b
Perillaldehyde <i>p</i> -Mentha-1,8-dien-7-al	2111-75-3	-	-	-	8.1	2025	0.54	8.1		Roberts et al., 2007
Balsam oil, Peru (<i>Myroxylon pereirae</i> Klotzsch)	8007-00-9	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	3.95	987	N/a	3.95		RIFM, 2004h
Peru balsam absolute	8007-00-9	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	2.5	625	N/a	2.5		RIFM, 2004i
Peru balsam absolute	8007-00-9	1:3 EtOH:DEP	0.5, 1.0, 2.5	4	>2.5	>625	N/a			RIFM, 2004i
Phenylacetaldehyde	122-78-1	4:1 AOO	2.5, 5, 10, 25, 50	4	3.0	750	0.25	3.0		Basketter et al., 2001

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

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

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

- = no data given; A216

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

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Chloroatranol

Chloroatranol (allergen in oak moss absolute: <i>Evernia prunastri</i>) (1)	
Design	blinded, randomised with regard to doses and controlled
Test subjects	13 patients previously identified as sensitized to chloroatranol and oak moss absolute
Controls	10 healthy controls
Substance	Purity: >99%
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	200 ppm to 0.0063 ppm (10 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction
ROAT	volar aspect of forearms
area	3 x 3 cm ²
applications/day	two
dose	chloroatranol in ethanol: Step 1: 5 ppm Step 2: 25 ppm
dose/application/cm ²	step 1: 0.025 µg step2: 0.125 µg
control substance	ethanol
definition of positive	erythema in at least 25% and at least one papule
period	two weeks for each step
Results	
PT ED10% (95% CI)	0.013 (0.002-0.03) ppm =0.0004 µg/cm ²
PT ED50% (95% CI)	0.15 (0.077-0.295) ppm =0.0045 µg/cm ²
PT no effect level (observed)	/
ROAT	Cumulative responses
Step 1 (5 ppm)	12/13 (92%)
Step 2 (25 ppm)	13/13 (100%)
Controls	Negative
Other information	None relevant

In a subsequent study chloroatranol and atranol, both ingredients in *Evernia prunastri*, were tested in equimolar concentrations in serial dilution in 10 eczema patients with known sensitization to chloroatranol and oak moss. A positive response was defined as any degree of reaction. Ethanol was included as the control and gave no response. No use tests were done and no control subjects included.

Results: All patients reacted to the highest concentrations of the two substances. For both substances there was a significant dose-dependence and the estimated difference in elicitation potency of chloroatranol relative to atranol was 217%. The dose-response curve is seen in figure 1 below (2).

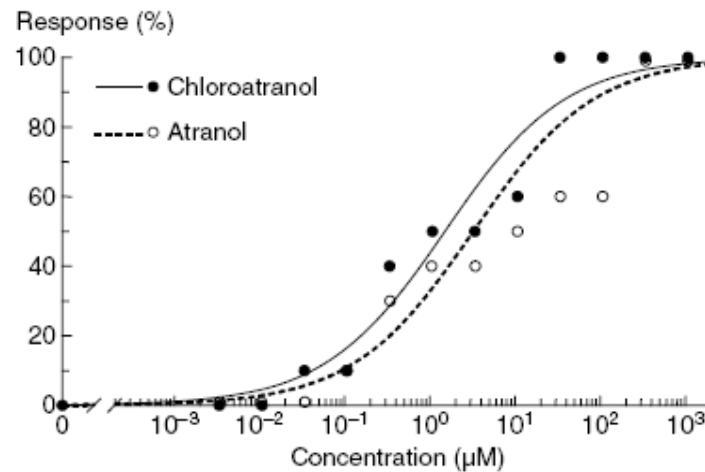


Fig. 1. Observed response rates and fitted parallel logistic dose-response curves for atranol and chloroatranol in equimolar concentrations at patch testing. The response was dichotomized and any reaction other than zero was classified as positive.

Cinnamal

Cinnamal (3)	
Design	blinded, randomised and controlled
Test subjects	18 patients with a positive patch test to cinnamal and additional 4 with a doubtful response
Controls	20 healthy controls
Substance	Purity: >98%
Patch test	20 mg solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2% to 0.01% (7 steps)
-control/vehicle	petrolatum
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of responses
ROAT	outer aspect of upper arm
area	5 x 5 cm ²
applications/day	two with atomizer pump
dose	Step 1: 0.02% Step 2: 0.1% Step 3: 0.8%
dose/application/cm ²	Not given
control substance	ethanol
definition of positive	The response was classified as positive no matter the degree of reaction.
period	two weeks for each step; total maximum 6 weeks
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	0.24% = 96 µg/cm ² (calculated from the data in the paper)
PT no effect level(observed)	0.01 % in pet. = 0.4 µg/cm ²
ROAT	Cumulative responses
Step 1 (0.02%)	0/18
Step 2 (0.1%)	8/18 (44 %)
Step 3 (0.8%)	13/18 (72 %)
Controls	No eczema reactions were seen
Other information	2 patients and 2 controls developed immediate reactions to the cinnamal solution

Cinnamal (4)	
Design	blinded, randomised doses and controlled
Test subjects	17 patients with a positive patch test to cinnamal (8 patients in part 1 and 9 in part two)
Controls	20 controls (non-sensitised dermatitis patients)
Substance	purity: /
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2 % to 0.00006 % (17 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration eliciting a + reaction
ROAT	Axilla
area	10 x 10 cm ² (estimated)
applications/day	two with roll on deodorant (89-700 mg per application of solution) average cases: 263 mg/application controls: only range given
dose	Part one: Step 1: 0.032% Step 2: 0.1% Step: 0.32% Part two: Step 1: 0.01% Step 2: 0.032% Step 3: 0.1%
dose/application/cm ²	Part two estimated: step one: 0.26 µg; step two: 0.84 µg; 2.63 µg
control substance	Deodorant matrix
definition of positive	eczematous reaction covering at least 25% of test area
period	Part one: one week with each concentration: maximum three weeks Part two: two weeks with each concentration: maximum six weeks
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	/
PT no effect level(observed)	0.002%
ROAT	Cumulative responses
Step 1 (0.01)	2/9 (22%)
Step 2 (0.032)	6/9 (67%)
Step 3 (0.1)	8/9 (88%)
Controls	No reactions were seen
Other information	Only reactions seen to the cinnamal-containing deodorants at ROAT, difference to matrix axilla ($p<0.001$) and all control

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	persons negative ($p < 0.001$)

Hydroxycitronellal

Hydroxycitronellal (5)	
Design	blinded, randomised doses and controlled
Test subjects	7 patients with a positive patch test to hydroxycitronellal
Controls	7 controls (non-sensitised dermatitis patients)
Substance	purity: /
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	4% to 0.00006% (17 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration eliciting + reaction
ROAT	Axilla
area	10 x 10 cm ² (estimated)
applications/day	two with roll on deodorant (172-591 per application of solution) average cases: 294 mg/application controls: only range given
dose	Step 1: 0.032% Step 2: 0.1% Step: 0.32%
dose/application/cm ²	Estimated: step 1: 0.94 µg; step 2: 2.94 µg; step 3: 9.40 µg
control substance	Deodorant matrix
definition of positive	eczematous reaction covering at least 25% of test area
period	two weeks with each concentration: maximum six weeks
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	/
PT no effect level(observed)	<0.00012 %
ROAT	Cumulative responses
Step 1 (0.032)	4/7 (57%)
Step 2 (0.1)	5/7 (71%)
Step 3 (0.32)	7/7 (100%)
Controls	No reactions were seen
Other information	Reactions were only seen to the hydroxycitronellal-containing deodorant at ROAT, difference to matrix treated axilla ($p<0.001$) and all control persons negative ($p<0.001$)

Hydroxycitronellal (6)	
Design	double blinded, randomised
Test subjects	13 patients with a positive patch test to hydroxycitronellal
Controls	/
Substance	purity: unknown
Patch test	confirmatory
-dilution steps	
-control/vehicle	
-definition of threshold	
ROAT	finger immersion in fragrance solution in 10% ethanol
area	/
applications/day	Once per day for 10 min
dose	Step 1: 10 ppm Step 2: 250 ppm
dose/application/cm ²	Not applicable
control substance	10% alcohol
definition of positive	clinical grading scale and laser doppler comparison between active and control
period	two weeks with each concentration: maximum four weeks
Results	
PT ED10% (95% CI)	Not relevant
PT ED50% (95% CI)	Not relevant
PT no effect level(observed)	Not relevant
ROAT	Cumulative responses
Step 1 (10 ppm)	1/13
Step 2 (250 ppm)	5/13
Vehicle control	4/13
Other information	No difference between active substance and control application was found.

Hydroxyisohexyl 3-cyclohexenecarboxaldehyde (HICC)

Hydroxyisohexyl 3-cyclohexenecarboxaldehyde (HICC) (7)	
Design	blinded, randomised and controlled
Test subjects	18 patients with a positive patch test to HICC
Controls	7 healthy controls
Substance	Purity: >99%
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	6% to 0.0006%
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of reactions
ROAT	volar aspect of lower arm
area	3 x 3 cm ²
applications/day	two with droplet bottle (theoretical:30 mg per application of solution)
dose	Step 1: 0.5% Step 2: 3%
µg/application/cm ²	Step 1: 15.3 (3.4-22.2) Step 2: 126.2 (40.5-226.2)
control substance	ethanol
definition of positive	erythema in at least 25% and at least one papule
period	two weeks for each step; total maximum 4 weeks
Results	
PT ED10% (95% CI)	0.9 µg/cm ² 29 (7-69) ppm
PT ED50% (95% CI)	20 µg/cm ² 662 (350-1250)ppm
PT no effect level (observed)	/
ROAT	Cumulative responses
Step 1 (0.5%)	11/18 (61%)
Step 2 (3%)	16/18 (89%)
Controls	No reactions were seen
Other information	Difference between test and control group statistically significant

Hydroxyisohexyl 3-cyclohexenecarboxaldehyde (HICC) (8)	
Design	blinded, randomised and controlled
Test subjects	15 patients with a positive patch test to HICC
Controls	10 healthy controls
Substance	Purity: > 98.8%
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	6% to 0.0006% (5 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of reactions
ROAT	Axilla
area	76 cm ² (template)
applications/day	two with roll on deodorant
dose	Step 1: 200 ppm Step 2: 600 ppm Step 3: 1800 ppm
dose/application/cm ²	median 0.79 µg HICC
control substance	deodorant matrix
definition of positive	spotty erythema involving at least 25% of the exposed area and infiltration represented by at least one papule.
period	two weeks for each step; total maximum 6 weeks
Results	
PT ED10% (95% CI)	0.75 µg/cm ² 25 ppm (0.69-120)
PT ED50% (95% CI)	18.3 µg/cm ² 610 ppm (120-2800)
PT no effect level (observed)	< 0.0006%
ROAT	Cumulative responses
Step 1 (200 ppm)	9/14* (64%)
Step 2 (600 ppm)	12/14* (86%)
Step 3 (1800 ppm)	14/14* (100%)
Controls	No reactions were seen
Other information	*14 patients completed the use test study Difference between HICC deodorant and matrix deodorant in cases ($p=0.0001$). Difference between controls and patients ($p=0.004$).

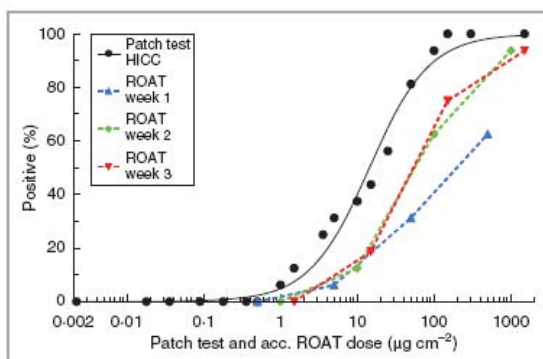
Hydroxyisohexyl 3-cyclohexenecarboxaldehyde (HICC) (9)	
Design	blinded, randomised and controlled
Test subjects	17 patients with a positive patch test to HICC
Controls	15 healthy controls
Substance	IFF lot SM/8059062
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	1500 to 0.0022 µg/cm ² HICC (19 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of reactions to higher concentrations
ROAT	volar aspect of forearms
area	3 x 3 cm (5 areas)
applications/day	two with micropipette (20 µl per application)
dose	Simultaneous application to 5 areas, four doses each and vehicle
µg /application/cm ²	Dose 1:0.0357 Dose 2: 0.357 Dose 3: 3.57 Dose 4: 35.7
control substance	ethanol
definition of positive	at least 5 points on a clinical scale, corresponding to erythema in 25% of test area and at least 1 papule
period	Three weeks. All concentrations applied simultaneously (randomised)
Results	
PT ED10% (95% CI)	0.662 µg/ cm ² (0.052-2.35)
PT ED50% (95% CI)	11.1 µg/ cm ² (3.41- 33.1)
PT no effect level(observed)	<0.0022 µg/ cm ²
ROAT	Cumulative responses
Dose 1 (0.0357)	0/16*
Dose 2 (0.357)	3/16 (19%)
Dose 3 (3.57)	12/16 (75%)
Dose 4 (35.7)	15/16 (94%)
Controls	No reactions were seen
Other information	*16 patients completed the use test study The evaporation rate of HICC was calculated to 72% over a 24-h period. ED10% ROAT: 0.064 µg/cm ² (more info see below)

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Table 2 The dose per application and accumulated dose after 1, 2 and 3 weeks in the ROAT

ROAT, dose per application ($\mu\text{g HICC cm}^{-2}$)	Number of applications after 1 week	Total accumulated dose after 1 week ($\mu\text{g HICC cm}^{-2}$)	Number of applications after 2 weeks	Total accumulated dose after 2 weeks ($\mu\text{g HICC cm}^{-2}$)	Number of applications after 3 weeks	Total accumulated dose after 3 weeks ($\mu\text{g HICC cm}^{-2}$)
35.7	14	500	28	1000	42	1500
3.57	14	50	28	100	42	150
0.357	14	5	28	10	42	15
0.0357	14	0.5	28	1	42	1.5

ROAT, repeated open application test; HICC, hydroxyisohexyl-3-cyclohexene carboxaldehyde.

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

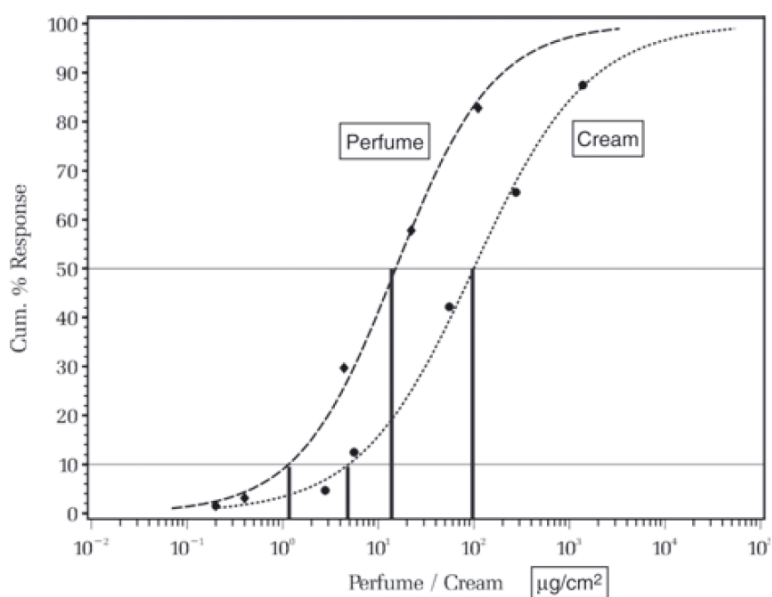
Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)

In a study by the German Contact Dermatitis Group, 64 persons previously diagnosed with HICC contact allergy were exposed to increasing doses of HICC in 2 different formulations, a hydrophilic cream and an ethanol solution, to mimic everyday exposures, following a standardised ROAT protocol (10). The concentration of HICC tolerated by 90% of the sensitised was estimated as 1.2 µg/cm² for perfume and 4.9 µg/cm² for cream. The dose-response curve is shown in Fig. 4.3 – 1 below.

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) (10)			
Design	randomised and vehicle controlled		
Test subjects	67 patients with a previous positive patch test to HICC		
Controls	None		
Substance	Provided by International Flavor & Fragrances Inc, Hilversum, NL		
Patch test			
-dilution steps	2.5% and 5%		
-control/vehicle	petrolatum		
-definition of threshold	lowest concentration giving a positive skin reaction in a continuous line to next higher concentration.		
ROAT	Volar forearms (both sides)		
area	3 x 3 cm (4 areas: one test and one control each for alcoholic solution and cream, respectively)		
applications/day	two		
dose	2.8 µg/cm ² in cream	0.2 µg/cm ² in ethanol	
	5.6 µg/cm ² in cream	0.4 µg/cm ² in ethanol	
	55.6 µg/cm ² in cream	4.4 µg/cm ² in ethanol	
	277.8 µg/cm ² in cream	22.2 µg/cm ² in ethanol	
	1388.9 µg/cm ² in cream	111.1 µg/cm ² in ethanol	
µg /application/cm ²	See above		
control substance	Ethanol 96% and glyceryl stearate 15% in water, resp.		
definition of positive	(spotty) erythema of at least 25% of the test area along with homogeneous infiltration or papules regardless of the number		
period	Two weeks for each step until positive reaction or end of study, whichever occurred first		
Results			
PT ED10% (95% CI)	Not calculable; 52 of 60 Patients patch tested positive to 2.5% HICC, 57 / 60 to 5% HICC		
PT ED50% (95% CI)	Not calculable		
PT no effect level (observed)	Not calculable		
ROAT	Cumulative responses:		
	Cream preparation: 2.8 µg/cm ² : 4.7%	Ethanol preparation: 0.2 µg/cm ² : 1.6%	

	5.6 $\mu\text{g}/\text{cm}^2$: 12.5% 55.6 $\mu\text{g}/\text{cm}^2$: 42.2% 277.8 $\mu\text{g}/\text{cm}^2$: 65.6% 1388.9 $\mu\text{g}/\text{cm}^2$: 87.5%	0.4 $\mu\text{g}/\text{cm}^2$: 3.1% 4.4 $\mu\text{g}/\text{cm}^2$: 29.7% 22.2 $\mu\text{g}/\text{cm}^2$: 57.8% 111.1 $\mu\text{g}/\text{cm}^2$: 82.8%
Controls	No reactions to vehicle in the patients included into analysis	
Other information	See figure below. Three patients were excluded from the study, so results are based on 64 patients.	

Figure 4.3 – 1: Dose-response curve of 64 patients sensitised to HICC, according to a previous PT, regarding two preparations: perfume and cream, the rhomboid and dot symbol, respectively, indicating the observed response. The curve was fitted by a logistic function (10).



Isoeugenol

Isoeugenol (11)	
Design	blinded, randomised doses and controlled
Test subjects	20 patients with a positive patch test to isoeugenol
Controls	20 healthy controls
Substance	purity: 98%
Patch test	20 mg solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2% to 0.01% (8 steps)
-control/vehicle	petrolatum
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line
ROAT	outer aspect of upper arms
area	5 x 5 cm (2 areas: one test and one control)
applications/day	two with roll-on
dose	0.2% in ethanol
μg /application/ cm^2	Doses measured to 0.14 -0.13 mg/application the first 14 days = 5.6 $\mu\text{g}/\text{cm}^2$
control substance	ethanol
definition of positive	any degree of reaction
period	Two weeks at upper arm and if negative another two weeks including application to base of neck
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	0.08% 32 $\mu\text{g}/\text{cm}^2$
PT no effect level (observed)	< 0.01% = 0.4 $\mu\text{g}/\text{cm}^2$
ROAT	
Dose: 0.2%	12/19 (63%)
Controls	No reactions were seen
Other information	

Isoeugenol (12)	
Design	blinded, randomised
Test subjects	27 patients with a positive patch test to isoeugenol
Controls	20 healthy controls
Substance	purity: 98%
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2% to 0.00006% (17 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of reactions to higher concentrations
ROAT	volar aspect of lower arm
area	3 x 3 cm (2 areas)
applications/day	two with droplet bottle (30 mg per application)
dose	0.05% in ethanol and 0.2%
µg /application/cm ²	Doses were calculated as mean 2.2 µg/cm ² (low conc.) and 9 µg/cm ² (high conc.)
control substance	ethanol
definition of positive	clear visible erythema
period	28 days
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	/
PT no effect level (observed)	< 0.0005% (5 ppm)
ROAT	Cumulative responses
Dose 1: 0.05%	10/24 (42%)
Dose 2: 0.2%	16/24 (67%)
Controls	No reactions were seen
Other information	Response to the low concentration in the ROAT appeared after median 15 days and to the high concentration after median 7 days.

Isoeugenol (13)	
Design	blinded, randomised and controlled
Test subjects	13 patients with a positive patch test to isoeugenol and 4 in part 1 (pre-test)
Controls	10 healthy controls (dermatitis patients)
Substance	purity: /
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2% to 0.00006% (w/v) (16 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration eliciting at least + reaction
ROAT	Axilla
area	10 x 10 cm ² (estimated)
applications/day	two with roll-on deodorant (117-586 mg per application of solution) average cases: 266 mg/application controls: only range given
dose	Part 1: Step 1:0.02% Step 2: 0.063% Step 3:0.2% Part 2: Step1:0.0063% Step 2:0.02% Step 3: 0.063%
dose/application/cm ²	Part 2: Step 1: 0.167 Step 2: 0.53 Step 3: 1.67 µg/application/cm ² (calculated based on data)
control substance	deodorant matrix
definition of positive	eczematous response covering 25% of test area
period	Part one: one week with each concentration: maximum three weeks Part two: two weeks with each concentration: maximum six weeks
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	/
PT no effect level (observed)	<0.0005% (0.15 µg/cm ²)
ROAT	
Step 1 (0.0063%)	3/13 (23%)
Step 2 (0.02%)	9/13 (69%)
Step 3 (0.063%)	10/13 (77%)
Controls	No reactions were seen
Other information	Deodorants containing cinnamal were responsible for all reactions in cinnamal sensitized individuals ($p<0.001$) and all control persons were negative ($p<0.001$)

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SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006

Version 6.2

Revision Date 17.09.2019

Print Date 21.10.2020

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 : Spearmint oil

Product Number : 60987

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. : 8008-79-5

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

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich Chimie Sarl
L'Isle D'Abeau Chesnes
F-38297 ST. QUENTIN FALLAVIER

Telephone : 0800 211408

Fax : 0800 031052

E-mail address : servicetechnique@merckgroup.com

1.4 Emergency telephone number

Emergency Phone # : +33 (0)9 75 18 14 07 (CHEMTREC)
+33 (0)1 45 42 59 59 (I.N.R.S.)

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008

Skin irritation (Category 2), H315

Eye irritation (Category 2), H319

Specific target organ toxicity - single exposure (Category 3), H335

For the full text of the H-Statements mentioned in this Section, see Section 16.

2.2 Label elements

Labelling according Regulation (EC) No 1272/2008

Pictogram



Signal word	Warning
Hazard statement(s)	
H315	Causes skin irritation.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.
Precautionary statement(s)	
P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Supplemental Hazard Statements	none

2.3 Other hazards - none

SECTION 3: Composition/information on ingredients

3.1 Substances

Synonyms : Mentha spicata

CAS-No. : 8008-79-5

Component	Classification	Concentration
Oils, spearmint		
	Skin Irrit. 2; Eye Irrit. 2; STOT SE 3; H315, H319, H335	<= 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

Consult a physician. Show this safety data sheet to the doctor in attendance.

If inhaled

If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact

Wash off with soap and plenty of water. Consult a physician.

In case of eye contact

Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

If swallowed

Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11



4.3 Indication of any immediate medical attention and special treatment needed
No data available

SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

5.2 Special hazards arising from the substance or mixture

Nature of decomposition products not known.

5.3 Advice for firefighters

Wear self-contained breathing apparatus for firefighting if necessary.

5.4 Further information

No data available

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures

Use personal protective equipment. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas.
For personal protection see section 8.

6.2 Environmental precautions

Do not let product enter drains.

6.3 Methods and materials for containment and cleaning up

Soak up with inert absorbent material and dispose of as hazardous waste. Keep in suitable, closed containers for disposal.

6.4 Reference to other sections

For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling

Avoid contact with skin and eyes. Avoid inhalation of vapour or mist.
Normal measures for preventive fire protection.
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities

Store in cool place. Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.

Recommended storage temperature 2 - 8 °C

Light sensitive.

7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated



SECTION 8: Exposure controls/personal protection

8.1 Control parameters

Components with workplace control parameters

8.2 Exposure controls

Appropriate engineering controls

Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

Personal protective equipment

Eye/face protection

Safety glasses with side-shields conforming to EN166 Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin protection

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

The selected protective gloves have to satisfy the specifications of Regulation (EU) 2016/425 and the standard EN 374 derived from it.

Full contact

Material: Nitrile rubber

Minimum layer thickness: 0,4 mm

Break through time: 480 min

Material tested: Camatril® (KCL 730 / Aldrich Z677442, Size M)

Splash contact

Material: Nitrile rubber

Minimum layer thickness: 0,11 mm

Break through time: 30 min

Material tested: Dermatril® (KCL 740 / Aldrich Z677272, Size M)

data source: KCL GmbH, D-36124 Eichenzell, phone +49 (0)6659 87300, e-mail sales@kcl.de, test method: EN374

If used in solution, or mixed with other substances, and under conditions which differ from EN 374, contact the supplier of the CE approved gloves. This recommendation is advisory only and must be evaluated by an industrial hygienist and safety officer familiar with the specific situation of anticipated use by our customers. It should not be construed as offering an approval for any specific use scenario.

Body Protection

Impervious clothing, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Respiratory protection

Where risk assessment shows air-purifying respirators are appropriate use a full-face respirator with multi-purpose combination (US) or type ABEK (EN 14387) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and



components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Control of environmental exposure

Do not let product enter drains.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

a) Appearance	Form: clear, liquid Colour: light yellow
b) Odour	No data available
c) Odour Threshold	No data available
d) pH	No data available
e) Melting point/freezing point	No data available
f) Initial boiling point and boiling range	228 °C - lit.
g) Flash point	94 °C - closed cup
h) Evaporation rate	No data available
i) Flammability (solid, gas)	No data available
j) Upper/lower flammability or explosive limits	No data available
k) Vapour pressure	No data available
l) Vapour density	No data available
m) Relative density	0,950 g/cm ³
n) Water solubility	No data available
o) Partition coefficient: n-octanol/water	No data available
p) Auto-ignition temperature	No data available
q) Decomposition temperature	No data available
r) Viscosity	No data available
s) Explosive properties	No data available
t) Oxidizing properties	No data available

9.2 Other safety information

No data available

SECTION 10: Stability and reactivity

10.1 Reactivity

No data available

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10.2 Chemical stability

Stable under recommended storage conditions.

10.3 Possibility of hazardous reactions

No data available

10.4 Conditions to avoid

No data available

10.5 Incompatible materials

Strong oxidizing agents

10.6 Hazardous decomposition products

Hazardous decomposition products formed under fire conditions. - Nature of decomposition products not known.

Other decomposition products - No data available

In the event of fire: see section 5

SECTION 11: Toxicological information

11.1 Information on toxicological effects

Acute toxicity

LD50 Oral - Rat - 5.000 mg/kg

Skin corrosion/irritation

Skin - Rabbit

Result: Skin irritation - 24 h

Serious eye damage/eye irritation

No data available

Respiratory or skin sensitisation

No data available

Germ cell mutagenicity

No data available

Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

Reproductive toxicity

No data available

Specific target organ toxicity - single exposure

Inhalation - May cause respiratory irritation.

Specific target organ toxicity - repeated exposure

No data available

Aspiration hazard

No data available

Additional Information

RTECS: WG7360000

Dermatitis, To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.



SECTION 12: Ecological information

12.1 Toxicity

No data available

12.2 Persistence and degradability

No data available

12.3 Bioaccumulative potential

No data available

12.4 Mobility in soil

No data available

12.5 Results of PBT and vPvB assessment

PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

12.6 Other adverse effects

No data available

SECTION 13: Disposal considerations

13.1 Waste treatment methods

Product

Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material.

Contaminated packaging

Dispose of as unused product.

SECTION 14: Transport information

14.1 UN number

ADR/RID: -

IMDG: -

IATA: -

14.2 UN proper shipping name

ADR/RID: Not dangerous goods

IMDG: Not dangerous goods

IATA: Not dangerous goods

14.3 Transport hazard class(es)

ADR/RID: -

IMDG: -

IATA: -

14.4 Packaging group

ADR/RID: -

IMDG: -

IATA: -

14.5 Environmental hazards

ADR/RID: no

IMDG Marine pollutant: no

IATA: no

14.6 Special precautions for user

No data available



SECTION 15: Regulatory information

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

This safety datasheet complies with the requirements of Regulation (EC) No. 1907/2006.

15.2 Chemical safety assessment

For this product a chemical safety assessment was not carried out

SECTION 16: Other information

Full text of H-Statements referred to under sections 2 and 3.

H315	Causes skin irritation.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.

Further information

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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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