Toxicological profile for

Peppermint absolute and or 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

8006-90-4: Oils, peppermint; Peppermint absolute (Mentha piperita); Mentha piperita oil; FEMA 2848; Oils, mentha piperita; Oil of peppermint; Peppermint oil; Euminz; HSDB 1900; IBgard; Mentharil; Peppermint oil (Mentha piperita); Peppermint oleoresin/extract (Mentha piperita L.); Peppermint terpenes; Pfefferminz oel [German]; UNII-AV092KU4JH (ChemIDplus); Essential oils, peppermint; Essential oils, Mentha piperita; CoE 282

84082-70-2: Extract of peppermint, EINECS 282-015-4; FEMA No. 2847; Peppermint leaves (Mentha piperita L.); Mentha piperita; Mentha piperita extract; Peppermint extract; Peppermint, ext. (ChemIDplus)

1.3. Molecular formula

Unspecified (ChemIDplus)

1.4. Structural Formula

No data available to us at this time.

1.5. Molecular weight (g/mol)

No data available to us at this time.

1.6. CAS registration number

8006-90-4, 84082-70-2

1.7. Properties

1.7.1. Melting point

(°C): 79.5 (CAS RN 8006-90-4) (EPISuite, 2017)

1.7.2. Boiling point

(°C): 216 (CAS RN 8006-90-4) (EPISuite, 2017)

1.7.3. Solubility

Very slightly soluble in water (CAS RN 8006-90-4) (Merck, 2013); 420-490 mg/L at 25°C (CAS RN 8006-90-4) (EPISuite, 2017)

1.7.4. pKa

No data available to us at this time.

1.7.5. Flashpoint

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

1.7.6. Flammability limits (vol/vol%)

No data available to us at this time.

1.7.7. (Auto)ignition temperature

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

1.7.8. Decomposition temperature

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

1.7.9. Stability

No data available to us at this time.

1.7.10. Vapor pressure

6.37E-02 mmHg at 25°C (CAS RN 8006-90-4) (EPISuite, 2017)

1.7.11. log Kow

3.19 or 3.40 (CAS RN 8006-90-4) (EPISuite, 2017)

2. General information

2.1. Exposure

Cosmetics	Yes (Cosmetics Bench Ref. 1996).	Food	Yes (Ash 1995; Burdock, 2010).
Environment	No evidence (Merck 2013).	Pharmaceuticals	Yes (Martindale 1993).

Peppermint (Mentha piperata) oil was detected in one depilatory product examined during the period 2000-2005 (Travassos et al. 2011).

Reported levels from use as a flavouring (ppm): (FEMA, 1994)

Food category	Usual	Max	Food category	Usual	Max
Alcoholic beverages	150.00	240.00	Gelatins, puddings	50.00	200.00
Baked goods	140.00	300.00	Meat products	6.00	8.00
Chewing gum	8300.00	8300.00	Nonalcoholic beverages	39.00	99.00
Confection, frosting	650.00	650.00	Soft candy	320.00	1200.00
Frozen dairy	95.00	110.00			

Estimated intake from flavouring use is: 1.1751 mg/kg bw/day.

As taken from Burdock, 2010.

Peppermint oil (CAS RN 8006-90-4) is listed as an ingredient in inside the home, personal care, pesticide and pet care products and peppermint extract (CAS RN 84082-70-2) as an ingredient in inside the home, personal care and pet care products by the US Department of Health and Human Services (2017).

Mentha piperita oil (CAS RN 8006-90-4/84082-70-2) is used as a masking, perfuming, refreshing and tonic ingredient, Mentha piperita extract as a cleansing, deodorant, masking, refreshing and tonic agent, Mentha piperita flower/leaf/stem extract as a flavouring, masking, perfuming and skin conditioning agent, Mentha piperita flower/leaf/stem water as a masking and perfuming agent, Mentha piperita herb extract as a perfuming agent, Mentha piperita leaf as a refreshing agent, Mentha piperita leaf extract, Mentha piperita leaf juice and Mentha piperita leaf water as skin conditioning agents and Mentha piperita water as a deodorant, masking, refreshing and tonic agent (all CAS RN 84082-70-2).

As taken from CosIng (Cosmetic substances and ingredients database). Available at http://ec.europa.eu/growth/tools-databases/cosing/ , accessed March 2018.

“Food: Spearmint oil and peppermint oil (usually rectified) 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 0.104% for peppermint oil in candy and about 0.132% (1318 ppm) for spearmint oil in baked goods.”

“Dietary Supplements/Health Foods: Leaves (or oil) of peppermint and spearmint, widely used as primary or adjunct flavoring for herb teas; capsules, tablets, tincture, and so on, in formulations for digestion, colds, and fevers (FOSTER).”

“Others: Peppermint oil and menthol are widely used in flavoring tobacco.”

As taken from Khan and Abourashed, 2010.

Peppermint oil, peppermint leaves, peppermint absolute, peppermint oil, rectified and peppermint

CO2 extract (all CAS RN 8006-90-4) are listed as fragrance ingredients by IFRA (2016), and oils, peppermint (CAS RN 8006-90-4) and peppermint (Mentha piperita) ext. (CAS RN 84082-70-2) on the US EPA Inert Finder Database (2018).

National Occupational Exposure Survey (1981 - 1983)
Estimated Numbers of Employees Potentially Exposed to Specific Agents by Occupation*

Agent Name	OIL, PEPPERMINT
CAS #	8006-90-4
RTECS #	SC6125000
Agent Code	80680

Code	Occupation Description (1980)	Total # Employees (Male & Female)	Total # Female Employees
019	MANAGERS AND ADMINISTRATORS, N.E.C.	986	
053	CIVIL ENGINEERS	483	88
095	REGISTERED NURSES	3,886	3,576
096	PHARMACISTS	529	349
099	OCCUPATIONAL THERAPISTS	300	197
103	PHYSICAL THERAPISTS	62	62
213	ELECTRICAL AND ELECTRONIC TECHNICIANS	17,319	8,562
216	ENGINEERING TECHNICIANS, N.E.C.	167	62
223	BIOLOGICAL TECHNICIANS	3,052	872
224	CHEMICAL TECHNICIANS	17	3
274	SALES WORKERS, OTHER COMMODITIES	1,315	1,315
363	PRODUCTION COORDINATORS	9	
365	STOCK AND INVENTORY CLERKS	464	
379	GENERAL OFFICE CLERKS	11	4
445	DENTAL ASSISTANTS	49	29
446	HEALTH AIDES, EXCEPT NURSING	427	176
447	NURSING AIDES, ORDERLIES, AND ATTENDANTS	111	61
449	MAIDS AND HOUSEMEN	1,853	1,447
453	JANITORS AND CLEANERS	1,526	
518	INDUSTRIAL MACHINERY REPAIRERS	91	
519	MACHINERY MAINTENANCE OCCUPATIONS	60	
529	TELEPHONE INSTALLERS AND REPAIRERS	6,749	
549	NOT SPECIFIED MECHANICS AND REPAIRERS	244	
575	ELECTRICIANS	59	
633	SUPERVISORS, PRODUCTION OCCUPATIONS	72	
634	TOOL AND DIE MAKERS	66	
637	MACHINISTS	5,923	268
674	MISCELLANEOUS PRECISION APPAREL AND FABRIC WORKERS	11	
684	MISCELLANEOUS PRECISION WORKERS, N.E.C.	1,924	
688	FOOD BATCHMAKERS	101	68
703	LATHE AND TURNING MACHINE SET-UP OPERATORS	28	
704	LATHE AND TURNING MACHINE OPERATORS	659	3
705	MILLING AND PLANING MACHINE OPERATORS	334	
708	DRILLING AND BORING MACHINE OPERATORS	749	
709	GRINDING, ABRADING, BUFFING, AND POLISHING MACHINE OPERATORS	1,548	71
715	MISCELLANEOUS METAL, PLASTIC, STONE, AND GLASS WORKING MACHINE OPERATORS	432	
719	MOLDING AND CASTING MACHINE OPERATORS	12	
734	PRINTING MACHINE OPERATORS	3,722	156
747	PRESSING MACHINE OPERATORS	657	657
748	LAUNDERING AND DRY CLEANING MACHINE OPERATORS	329	329
754	PACKAGING AND FILLING MACHINE OPERATORS	1,030	411

755	EXTRUDING AND FORMING MACHINE OPERATORS	101	68
756	MIXING AND BLENDING MACHINE OPERATORS	634	
759	PAINTING AND PAINT SPRAYING MACHINE OPERATORS	101	68
774	PHOTOGRAPHIC PROCESS MACHINE OPERATORS	657	657
777	MISCELLANEOUS MACHINE OPERATORS, N.E.C.	2,557	186
779	MACHINE OPERATORS, NOT SPECIFIED	2,056	441
785	ASSEMBLERS	956	
796	PRODUCTION INSPECTORS, CHECKERS, AND EXAMINERS	452	329
797	PRODUCTION TESTERS	68	68
804	TRUCK DRIVERS, HEAVY	197	
877	STOCK HANDLERS AND BAGGERS	138	
887	VEHICLE WASHERS AND EQUIPMENT CLEANERS	23	
888	HAND PACKERS AND PACKAGERS	617	617
889	LABORERS, EXCEPT CONSTRUCTION	1,124	430
TOTAL		67,045	21,627

*(1) The estimates for each occupation apply across the surveyed industries in which the agent was observed. Not all industries were surveyed, and not all agents were observed in all surveyed industries. (2) When using the estimates, standard errors associated with estimates should be considered. (3) Potential exposures to a chemical agent are categorized as actual (i.e., the surveyor observed the use of the specific agent) or tradename (i.e., the surveyor observed the use of a tradename product known to contain the specific agent). The estimates presented in the table combine both categories.

As taken from NIOSH, available at https://web.archive.org/web/20111028111422/http://www.cdc.gov/noes/noes2/80680occ.html

2.2. Combustion products

This ingredient was investigated in a pyrolysis study. Results are given in JTI Study Report (s).

Compound	Two stage heating		One stage heating	
	Abundance	Area%	Abundance	Area%
ethanol + acetone	304295156	1.83	464990713	1.73
limonene	374610636	2.25	747662121	2.78
1,8-cineole + p-cymene	1037765626	6.24	1507107686	5.60
gamma-terpinene	210928965	1.27	424974602	1.58
3-octanol	113915547	0.69	273211709	1.01
trans-sabinene hydrate + pentyl isovalerate	401633213	2.42	949370138	3.53
menthofuran	664589762	4.00	1477416426	5.49
menthone	2711756829	16.31	3906938994	14.51
neomenthol	805089759	4.84	1089878769	4.05
terpinen-4-ol + isomenthone	956473960	5.75	884692490	3.29
menthol	4052050876	24.37	5053219826	18.76
alpha terpineol + menthol isomer	220866262	1.33	357229464	1.33
pulegone + unknown	464532121	2.79	951821329	3.53
menthyl acetate + carvone	1146427266	6.90	1979659083	7.35
piperitone	269659796	1.62	560409713	2.08
beta-bourbonene	175671868	1.06	404832477	1.50
beta-caryophyllene	517050243	3.11	1066880336	3.96
germacrene d	228400197	1.37	622693875	2.31

Total ion chromatogram	16671547226	100	26956444805	100

This ingredient was investigated in a pyrolysis study. Results are given in Baker and Bishop (2005) J. Anal. Appl. Pyrolysis 74, 145–170.

Ingredient Name & CAS Number	Max. cig. appln. level (ppm)	Purity of Sample (%)	Composition of pyrolysate (Compound, %)	Max. level in smoke (mg)
Peppermint oil 8006-90-4	35	na	Menthol (44.9) Menthone (19.9) Menthofuran (10.4) Menthol acetate (5.8) Cineole (4.6)	8 3 2 1 0.8

2.3. Ingredient(s) from which it originates

Peppermint oil derived from Mentha piperita, and cornmint and peppermint oil derived from Menta arvensis/ [BUREAU OF THE CENSUS. U.S. IMPORTS FOR CONSUMPTION AND GENERAL IMPORTS 1984 p.1-374 and 1-375] **PEER REVIEWED**

As taken from HSDB, 2003

Peppermint oil is obtained by steam distillation of the fresh, overground parts of the flowering plant, Mentha piperita.

As taken from Burdock, 2010.

No evidence of its presence in tobacco naturally (Stedman 1968; Lloyd 1976).

“Peppermint yields 0.1–1.0% (usually 0.3–0.4%) of volatile oil…..”

“Spearmint yields normally about 0.7% volatile oil, …….”

“Cornmint contains 1–2% volatile oil …..”

As taken from Khan and Abourashed, 2010.

Mentha piperita oil (CAS RN 8006-90-4/84082-70-2) is the volatile oil and Mentha piperita extract (CAS RN 84082-70-2) is an extract obtained from the whole plant of the peppermint, Mentha piperita (L.), Labiatae.

Mentha piperita flower/leaf/stem extract is an extract and Mentha piperita flower/leaf/stem water is the aqueous solution of the steam distillates (both CAS RN 84082-70-2) of the flowers, leaves and stems of the peppermint, Mentha piperita (L.), Labiatae.

Mentha piperita herb extract (CAS RN 84082-70-2) is an extract obtained from the herbs of the peppermint, Mentha piperita (L.), Labiatae.

Mentha piperita leaf is the leaves, Mentha piperita leaf extract is an extract of the leaves, Mentha piperita leaf juice is the juice expressed from the leaves and Mentha piperita leaf water is an aqueous solution of the steam distillate (all CAS RN 84082-70-2) obtained from the leaves of the peppermint, Mentha piperita (L.), Labiatae.

As taken from CosIng (Cosmetic substances and ingredients database). Available at http://ec.europa.eu/growth/tools-databases/cosing/, accessed March 2018.

3. Status in legislation and other official guidance

Essential oils, oleoresins (solvent-free), and natural extractives (including distillates) that are generally recognized as safe for their intended use, within the meaning of section 409 of the Act. Peppermint is included on this list. [21 CFR 182.20 (4/1/97)] **PEER REVIEWED**

As taken from HSDB, 2003

States approving use in tobacco	UK, France, Germany, Belgium					
Food	UK	Yes	EU	Yes	USA	Yes
ADI	Not listed					
Codex Alim.	Not listed					
C of E no.	282			FEMA no.	2848	
TLV (ACGIH)	Not listed					
Cosmetics (UK)	Not listed in Schedule 1					

Peppermint, oil (Mentha piperita L. (CAS RN 8006-90-4) is included on the US FDA's list of Everything Added to Food in the United States (EAFUS) as GRAS (Generally Recognized as Safe) under 21 CFR section 182.20 (Essential oils, oleoresins (solvent-free), and natural extractives (including distillates)). It is also included under 21 CFR section 172.230 (microcapsules for flavoring substances) (EAFUS, 2013; FDA, 2018)

Peppermint oil, peppermint leaves, peppermint absolute, peppermint oil, rectified and peppermint CO2 extract (all CAS RN 8006-90-4) are listed by IFRA (2016).

Peppermint oil (Mentha piperita) appears on the list of "Permitted Additives to Tobacco Products in the United Kingdom" (Department of Health, 2003) at a maximum level permitted for inclusion in cigarettes/RYO, cigars and pipe tobacco of 2 % w/w.

Oils, peppermint (CAS RN 8006-0-4) are pre-registered under REACH ("envisaged registration deadline 30 November 2010") (ECHA, 2018a).

There is a REACH dossier on peppermint extract (CAS RNs 8006-90-4/84082-70-2) (ECHA, 2018b).

Oils, peppermint (CAS RN 8006-90-4) are listed in the US EPA Inert Finder Database (2018) as approved for non-food and fragrance use pesticide products and peppermint (Mentha piperita) ext. (CAS RN 84082-70-2) for fragrance use pesticide products.

Oils, peppermint (CAS RN 8006-90-4) are listed in the US EPA Toxic Substances Control Act (TSCA) inventory and also in the US EPA CDR list (Chemical Data Reporting Rule). The Chemical Data Reporting (CDR) Rule requires companies that manufacture (including import) certain chemicals at certain volumes in the U.S. to report to EPA every four years through its CDR.

The TSCA inventory and 2012 CDR list are available at https://iaspub.epa.gov/sor_internet/registry/substreg/searchandretrieve/searchbylist/search.do

"The highest recommended daily dose in the EU is 1.2 ml peppermint oil i.e. 1080-1099 mg peppermint oil (based on relative density 0.9-0.916 g/cm^3 according Ph. Eur. 8.1 (2014)" (EMA, 2014).

Neither CAS RN is classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2018c).

Peppermint extract (CAS RN 84082-70-2) and oils, peppermint (CAS RN 8006-90-4) are listed in the New Zealand Inventory of Chemicals; peppermint extract being allowed for use as a single component chemical under an appropriate group standard, and oils, peppermint with HSNO

Approval Code HSR003779 (NZ EPA, 2006) and being classified according to the New Zealand authorities (NZ EPA CCID).

Peppermint oil (CAS RN 8006-90-4) and peppermint leaves (Mentha piperita L.) (CAS RN 84082-70-2) were granted GRAS status for use as food flavourings in the US by FEMA (Hall amd Oser, 1965).

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

"The major biliary metabolite is menthol glucuronide, which undergoes enterohepatic circulation. The urinary metabolites result from hydroxylation at the C-7 methyl group at C-8 and C-9 of the isopropyl moiety, forming a series of mono- and dihydroxymenthols and carboxylic acids, some of which are excreted in part as glucuronic acid conjugates."

As taken from Grigoleit HG & Grigoleit P. Phytomedicine. 2005 Aug; 12(8):612-6. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=16121523&query_hl=19&itool=pubmed_DocSum

"OBJECTIVE: Peppermint oil (PMO) has been used to treat abdominal ailments dating to ancient Egypt, Greece and Rome. Despite its increasing paediatric use, as in irritable bowel syndrome (IBS) treatment, the pharmacokinetics (PK) of menthol in children given PMO has not been explored. DESIGN AND SETTING: Single-site, exploratory pilot study of menthol PK following a single 187 mg dose of PMO. Subjects with paediatric Rome II defined (IBS; n=6, male and female, 7-15 years of age) were enrolled. Blood samples were obtained before PMO administration and at 10 discrete time points over a 12 h postdose period. Menthol was quantitated from plasma using a validated gas chromatography mass spectrometry technique. Menthol PK parameters were determined using a standard non-compartmental approach. RESULTS: Following a dose of PMO, a substantial lag time (range 1-4 h) was seen in all subjects for the appearance of menthol which in turn, produced a delayed time of peak (Tmax=5.3 ± 2.4 h) plasma concentration (Cmax=698.2 ± 245.4 ng/mL). Tmax and Tlag were significantly more variable than the two exposure parameters; Cmax, mean residence time and total area under the curve (AUC=4039.7 ± 583.8 ng/mL × h) which had a coefficient of variation of <20%. CONCLUSIONS: Delayed appearance of menthol in plasma after oral PMO administration in children is likely a formulation-specific event which, in IBS, could increase intestinal residence time of the active ingredient. Our data also demonstrate the feasibility of using menthol PK in children with IBS to support definitive studies of PMO dose-effect relationships." As taken from Kearns GL et al. 2015. BMJ Open 5(8), e008375. PubMed, 2016 available at: http://www.ncbi.nlm.nih.gov/pubmed/26270949

4.2. Absorption, distribution and excretion

Peppermint oil was absorbed through the skin of mice, and was detected in the blood one hour after dermal application (BIBRA, 1999).

When human volunteers were administered orally 180 mg of the oil in a capsule, 20-65% of the dose was excreted in the urine within 14 hours (BIBRA, 1999).

"Pharmacokinetic studies reveal that fractionated urinary recovery of menthol is dependent on the kind of formulation used for the application of PO. Optimal pH triggered enteric coated formulations start releasing PO in the small intestine extending release over 10-12 h thus providing PO to the target organ in irritable bowel syndrome, i.e. the colon."

As taken from Grigoleit HG & Grigoleit P. Phytomedicine. 2005 Aug; 12(8):607-11. PubMed, 2010

available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=16121522&query_hl=19&itool=pubmed_DocSum

"In animals, peppermint is rapidly absorbed. The major biliary metabolite is menthol glucuronide, which undergoes enterohepatic circulation. After inhalation, pulmonary absorption depends on various factors and the rapid elimination indicates that there should be no accumulation during long-term application.
The urinary metabolites are excreted in part as glucuronic acid conjugates. Studies in rats indicated equal excretion in feces and urine of essential oil compounds. The main metabolite identified was menthol-glucuronide" (EMEA, 2008).

"A randomized, two-way, crossover, bioequivalence study in 6 beagle dogs was conducted to compare the bioavailability of two peppermint oil formulations, soft capsule and hard capsule. The drug was given in a single dose of two capsules (total, 200 mg), and blood samples were withdrawn during the 12 h after drug administration. Menthol (CAS 2216-51-5) as the main component of peppermint oil was determined by a gas chromatography-tandem mass spectrometry (GC-MS/I MS) method after cleavage with beta-glucuronidase. The following pharmacokinetic variables were computed for the two formulations: maximum concentration (Cmax), time to maximum concentration (Tmax), half-life of elimination (t1/2), mean residence time (MRT), and areas under the plasma concentration-time curve (AUC(0-t) and AUC(0-infinity)). For calculation of the 90% confidence interval (CI), an analysis of variance (ANOVA) was carried out. The results indicated that treatment and subject had statistically significant effect on AUC(0-t), AUC(0-infinity), and Cmax, and the 90% CIs for AUC(0-t), AUC(0-infinity), and Cmax were outside the acceptable bioequivalence range. The relative bioavailability was 121.4 +/- 10.6% for AUC(0-infinity). Therefore, it can be concluded that the two formulations are not bioequivalent and the bioavailability of soft capsules is significantly higher than that of hard capsules" (Wu et al. 2010).

4.3. Interactions

"The principal pharmacodynamic effect of peppermint oil relevant to the gastrointestinal tract is a dose-related antispasmodic effect on the smooth musculature due to the interference of menthol with the movement of calcium across the cell membrane."

As taken from Grigoleit HG & Grigoleit P. Phytomedicine. 2005 Aug; 12(8):612-6. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=16121523&query_hl=19&itool=pubmed_DocSum

"Exposure to environmental stresses and toxins is linked to the pathogenesis of neuropsychiatric disorders. Astrocytes, the most abundant glial-cell type in the brain, are considered to have physiological and pathological roles in neuronal activities. We have investigated whether peppermint oil inhibits heat shock-induced apoptosis of astrocytes. We found that peppermint oil inhibits the heat shock-induced apoptosis in both human astrocyte CCF-STTG1 cells and rat astrocytes. Pretreatment of the cells with peppermint oil inhibited the heat shock-induced DNA fragmentation and condensation of nuclear chromatin. Peppermint oil also inhibited the caspase-3 activation and poly-ADP-ribose polymerase fragmentation in CCF-STTG1 cells. These results suggest that peppermint oil may modulate the apoptosis of astrocytes via the activation of the caspase-3."

As taken from Koo HN et al. J Mol Neurosci. 2001 Dec; 17(3):391-6. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=11859935&query_hl=19&itool=pubmed_DocSum

"The influence of peppermint oil on intestinal transport was investigated in rat jejunum using both intestinal sheets mounted in Ussing chambers and brush border membrane vesicles. Mucosal peppermint oil (1 and 5 mg/ml) had no significant effect on basal short circuit current, but inhibited

the increase associated with sodium dependent glucose absorption. The increased short circuit current induced by serosal acetylcholine, a reflection of calcium mediated electrogenic chloride secretion, was unaffected by mucosal peppermint oil (5 mg/ml). In contrast, serosal peppermint oil (1 mg/ml) inhibited the response to acetylcholine without reducing the effect of mucosal glucose. In brush border membrane vesicles active glucose uptake was inhibited by extravesicular peppermint oil at concentrations of 0.5 and 1 mg/ml. Peppermint oil in the intestinal lumen inhibits enterocyte glucose uptake via a direct action at the brush border membrane. Inhibition of secretion by serosal peppermint oil is consistent with a reduced availability of calcium."

As taken from Beesley A et al. Gut. 1996 Aug; 39(2):214-9. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=8991859&query_hl=19&itool=pubmed_DocSum

"The appearance of common and self-initiative usage of various herbal preparations in everyday practice and life imposes the question of possible interactions with drugs. This survey examined the influence of acute and chronic peppermint oil (PO--Mentha × piperita L., Lamiaceae; prepared as emulsion for oral use) on pentobarbitone-induced sleeping time, analgesic effect of codeine and impairment of motor coordination caused by midazolam in mice. The chemical profile of essential oil was determined by GC-MS. Applied doses of PO were 0.1 and 0.2 mL/kg. Chronic PO intake (in both doses) led to significant decrease of analgesic effect of codeine, while acute intake of PO did not change this effect. Acute PO pretreatment in higher dose caused significant prolongation of pentobarbitone-induced sleeping time, while it was significantly shortened by chronic PO pretreatment at the same dose. Midazolam effect was enhanced and prolonged significantly by chronic PO intake at higher dose, while acute intake of PO did not change this effect. Gut motility was increased only by acute intake of higher PO dose. Regarding the fact that PO produces changes in tested drug effects, the interaction between drugs and phytopreparations containing PO should be additionally followed/confirmed in humans." As taken from Samojlik I et al. 2012. Phytother. Res. 26 (6), 820-5. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22076909?dopt=AbstractPlus

"In this study we investigated the relationship between several selected commercially available essential oils and beta-lactam antibiotics on their antibacterial effect against multidrug resistant bacteria. The antibacterial activity of essential oils and antibiotics was assessed using broth microdilution. The combined effects between essential oils of cinnamon bark, lavender, marjoram, tea tree, peppermint and ampicillin, piperacillin, cefazolin, cefuroxime, carbenicillin, ceftazidime, meropenem, were evaluated by means of the checkerboard method against beta-lactamase-producing Escherichia coli. In the latter assays, fractional inhibitory concentration (FIC) values were calculated to characterize interaction between the combinations. Substantial susceptibility of the bacteria toward natural antibiotics and a considerable reduction in the minimum inhibitory concentrations (MIC) of the antibiotics were noted in some paired combinations of antibiotics and essential oils. Out of 35 antibiotic-essential oil pairs tested, four of them showed synergistic effect (FIC≤0.5) and 31 pairs showed no interaction (FIC>0.5-4.0). The preliminary results obtained highlighted the occurrence of a pronounced synergistic relationship between piperacillin/cinnamon bark oil, piperacillin/lavender oil, piperacillin/peppermint oil as well as meropenem/peppermint oil against two of the three bacteria under study with a FIC index in the range 0.26-0.5. The finding highlighted the potential of peppermint, cinnamon bark and lavender essential oils being as antibiotic resistance modifying agent. Reduced usage of antibiotics could be employed as a treatment strategy to decrease the adverse effects and possibly to reverse the beta-lactam antibiotic resistance." As taken from Yap PS et al. 2013. Phytomedicine 20(8-9), 710-3. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23537749

"CONTEXT: Plant extracts are commonly used in a number of cosmetics and topical pharmaceuticals. The effects on such extracts on the subsequent dermal absorption and penetration of other cosmetic ingredients needs to be evaluated. OBJECTIVE: This study demonstrates the effect of some natural extracts routinely found in cosmetics on the dermal absorption and penetration of marker penetrants. METHODS: Aqueous ethanolic extracts of Gingko biloba, Lavendula angustifolia, Rosmarinus officinale, Mentha piperita, Matricaria recutita, Persea

Americana, Avena sativa, Zingiber officinale were prepared. 14C-caffeine and 14C-salicylic acid were topically dosed with either 10% solutions of natural extracts or ethanol (control) using a flow through in vitro porcine skin diffusion system. Samples were analyzed with liquid scintillation counter. The parameters of flux, permeability, and percent dose absorbed/retained were calculated and compared. RESULTS: The dermal absorption of 14C-caffeine was significantly higher ($p \geq 0.05$) with avocado, chamomile, ginger and peppermint extract as compared to the control ethanol; while dermal absorption of 14C-salicylic acid was significantly greater with ginkgo and chamomile extract as compared to ethanol. Over four fold increase in flux and permeability of caffeine with avocado extract was observed while chamomile and peppermint extracts increased the flux and permeability of caffeine over three fold. Gingko and chamomile extracts increased salicylic acid's flux and permeability by two fold. Sum of %dose skin residue + %absorption in receptor fluid for different extracts exhibited the similar trend as shown by flux and permeability. The other natural extracts tested did not produce statistically significant effects on dermal penetration parameters for both caffeine and salicylic acid ($p \geq 0.05$). CONCLUSION: These results emphasize the influence of natural plant extracts on the transdermal penetration of hydrophilic (caffeine) and hydrophobic (salicylic acid) penetrants and thus warrants the consideration as to their safety in cosmetics and topical pharmaceuticals containing natural extracts." As taken from Muhammad F et al. 2017. Cutan. Ocul. Toxicol. 36(1), 60-66. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27027912

5. Toxicity

5.1. Single dose toxicity

Record for CAS RN 8006-90-4:

Organism	Test Type	Route	Reported Dose (Normalized Dose)	Effect	Source
mouse	LD50	oral	2490mg/kg (2490mg/kg)		Tokishikoroji Foramu. Toxicology Forum. Vol. 8, Pg. 91, 1985.
rat	LD50	intraperitoneal	819mg/kg (819mg/kg)	BEHAVIORAL: CONVULSIONS OR EFFECT ON SEIZURE THRESHOLD BEHAVIORAL: ATAXIA LUNGS, THORAX, OR RESPIRATION: RESPIRATORY DEPRESSION	Journal of Pharmaceutical Sciences. Vol. 54, Pg. 1071, 1965.
rat	LD50	oral	2426mg/kg (2426mg/kg)	BEHAVIORAL: ATAXIA BEHAVIORAL: MUSCLE CONTRACTION OR SPASTICITY) LUNGS, THORAX, OR RESPIRATION: RESPIRATORY DEPRESSION	Journal of Pharmaceutical Sciences. Vol. 54, Pg. 1071, 1965.

As taken from ChemIDplus, available at https://chem.nlm.nih.gov/chemidplus/

Peppermint oil is of low oral toxicity

Record for CAS RN 8006-90-4:

Species	Route	Dose data	Toxic effects	References
Rat	Oral	LD_{50}: 2426 mg/kg bw	Behavioral - ataxia Behavioral - muscle contraction or spasticity Lungs, Thorax, or Respiration - respiratory depression	JPMSAE Journal of Pharmaceutical Sciences. (American Pharmaceutical Assoc., 2215 Constitution Ave., NW, Washington, DC 20037) V.50- 1961- Volume(issue)/page/year: 54,1071,1965
Rat	Oral	TDLo: 0.83 mL/kg	Liver - change in gall bladder structure or function	CEXPB9 Clinical and Experimental Pharmacology and Physiology. (Blackwell Scientific Publications, (Australia) Pty Ltd., 107 Barry St., Carlton, Vic. 3053, Australia) V.1- 1974- Volume(issue)/page/year: 30,799,2003
Rat	Intraperitoneal	LD_{50}: 819 mg/kg bw	Behavioral - convulsions or effect on seizure threshold Behavioral - ataxia Lungs, Thorax, or Respiration - respiratory depression	JPMSAE Journal of Pharmaceutical Sciences. (American Pharmaceutical Assoc., 2215 Constitution Ave., NW, Washington, DC 20037) V.50- 1961- Volume(issue)/page/year: 54,1071,1965
Mouse	Oral	LD_{50}: 2490 mg/kg bw		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, 2011

“Atrial fibrillation, muscle pain, cooling sensation, burning sensation after acute exposure.”

“ANIMALS SHOWED MILD STIMULATION FOLLOWED BY DEPRESSION, TWITCHING, SPASTIC CONVULSIONS, ATAXIA, HINDLIMB PARALYSIS & SLOWED RESPIRATIONS.”

As taken from HSDB, 2003

“A man who consumed a “peppermint capsule” to relieve his diarrhoea felt within 3 hr a severe burning sensation in the anus on defecation [no information on the quantity of peppermint oil in the capsule was given] (Weston, 1987).” (BIBRA, 1999)

“Nausea and the urge to urinate was reported in one of five volunteers who had 0.1 ml peppermint oil (dissolved in 20 ml saline) instilled into their large bowel. Four experienced abdominal cramps, pain and the urge to defecate (Rogers et al. 1988).” (BIBRA, 1999)

LD50 oral, rat – 2426mg/kg (Ash 1995)

LD50 intraperitoneal, rat - 819mg/kg (Ash 1995)

5.2. Repeated dose toxicity

A daily dose of up to 1.2 ml peppermint oil, in capsules, was well tolerated by patients with irritable bowel syndrome in a three week clinical trial. Heartburn was observed in two individuals, but the

investigators deduced that this could have been caused by premature release of the oil into the stomach (BIBRA, 1999).

Peppermint oil was investigated in a number of repeated dose studies (BIBRA, 1999). No overt clinical signs of toxicity were observed in rats given up to 500 mg/kg bw/day for five weeks by stomach tube. Furthermore, no treatment related changes were observed at post mortem. Previous similar studies, both 28 and 90 day exposures, had resulted in a no-observed effect level being established at 40 mg/kg bw/day, due to microscopic brain lesions. However, a subsequent review concluded that these lesions were artefacts resulting from inadequate preparation of the tissue prior to examination.

“Similar effects were reported from the same laboratory in a study that followed the same protocol, when peppermint oil containing 1–3% R(+)-pulegone was given by gavage to provide a dose of 0, 10, 40, or 100 mg/kg bw per day to groups of 10 male and of10 female Wistar SPF rats for 28 days. No differences in body weight or food consumption were found between treated and control groups. A slight, non-significant increase in water consumption was reported in all treated groups. Haematological examinations, blood chemical determinations, and urine analysis revealed normal values. The only significant histopathological change was the appearance of ‘cyst-like spaces’ in the white matter of the cerebellum in animals at the two higher doses, but there were no obvious clinical sugns of encephalopathy (Thorup et al., 1983b; Olsen & Thorup, 1984).”

“Peppermint oil containing 1–2% pulegone was administered to groups of three beagle dogs of each sex at a dose of 25 or 125 mg/kg bw per day or to groups of 12 male Wistar rats at a dose of 20, 150, or 500 mg/kg bw per day, by gavage for 5 weeks. The animals were inspected daily for clinical signs; body weight and food consumption were recorded weekly; haematological, blood biochemical, and urinary parameters were measured before treatment and during week 5; and histological examination was conducted at termination. The rats showed no effects on general health, behaviour, or body weight, and the hematological and urinary parameters were normal. Histological examination revealed no specific pathological lesions. A reduction in triglyceride concentration in rats at the high dose was attributed to decreased food consumption. Similar results were found for dogs, except that males at the high dose had slightly, non-significantly increased alkaline phosphatase activity and urea concentrations. These increases were considered to be of no toxicological relevance (Mengs & Stotzem, 1989).”

“Peppermint oil containing 1.1% pulegone was administered to groups of 14 male and 14 female Wistar rats by oral gavage in soya bean oil at a dose of 0, 10, 40, or 100 mg/kg bw per day for 90 days. Body weights and food and water consumption were measured weekly; no differences were found between treated and control animals. Haematological examinations and blood chemical determinations perfomed on 10 animals of each sex on days 30 and 86 of dosing showed normal values. Animals at the low and intermediate doses showed no effects, but male rats at the high dose had nephropathy, in the form of hyaline droplets. The authors concluded that this effect was an early manifestation of sex- and species-specific nephropathy due to the appearance of *alpha*-2-microglobulin in the kidney. ‘Cyst-like spaces’ in the cerebellum were reported in animals at the high dose, but there were no other signs of encephalopathy (Spindler & Madsen, 1992). As this effect was not reproduced in the 28-day study in which animals were given pulegone at 160 mg/kg bw per day, an NOEL for peppermint oil of 40 mg/kg bw per day could be identified, which corres-ponds to an NOEL for pulegone of 0.44 mg/kg bw per day. Nevertheless, it is ques-tionable whether the effects at the high dose are relevant in terms of human risk.”

As taken from WHO Food Additives Series 46, available at http://www.inchem.org/documents/jecfa/jecmono/v46je10.htm

“1. The aim of the present study was to investigate the effects of peppermint oil and valerian on rat liver and cultured human hepatoma cells. 2. Rats received a single oral dose of peppermint oil (8.3-830 microL/kg) or valerian (0.31-18.6 g/kg), or daily oral doses of 83 microL/kg peppermint oil or 3.1 g/kg valerian for 28 days. After 24 h, rats were anaesthetized and measurements made of bile flow, liver function and in vivo sinusoidal area. Livers were then removed for histology. 3. Bile flow was unaffected by any treatment, except acute high-dose peppermint oil (830 microL/kg; 70% increase

in flow). No change in liver enzyme activity was found, except for a 45% increase in alkaline phosphatase after chronic peppermint oil. No change in sinusoidal area in vivo or in histology was found following any treatment, although pretreatment with carbon tetrachloride reduced sinusoidal bed area and produced histological damage. Incubation of human hepatoma cells with 0.5 microL/mL (but not 0.05 microL/mL) peppermint oil or 20 mg/mL (but not 2 mg/mL) valerian resulted in increased cell death. 4. In conclusion, the present study demonstrated in vitro toxicity of high doses of valerian and peppermint oil in cultured human hepatoma cells and, at doses 2-3 orders of magnitude greater than those recommended for human use, an increase in rat bile flow after acute peppermint oil and an increase in alkaline phosphatase after chronic peppermint oil."

As taken from Vo LT et al., (2003), Clin Exp Pharmacol Physiol. 2003 Oct; 30(10):799-804. Pubmed, 2011 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=14516421&query_hl=3&itool=pubmed_docsum

"An English abstract of a Danish paper reports burning sensations in the throat and stomach amongst 19 patients with irritable bowel syndrome who took part in a clinical trial of capsules containing 200 mg peppermint oil [at unspecified concentrations]. Four capsules each containing 50 mg peppermint oil were consumed 30 minutes prior to each meal (Lech et al. 1988). In an equivalent Swedish clinical trial, four of 30 patients reported dry mouth when they took 0.6 ml peppermint oil daily in capsules (Carling et al. 1989)." (BIBRA, 1999)

"In a 4-wk clinical trial involving daily doses of 90 mg peppermint oil (and 50 mg caraway oil) in capsular form, there was one adverse reaction (out of 19 patients with non-ulcer dyspepsia) which the investigators believed was treatment-related, a substernal burning sensation with severe eructation [belching] and nausea. Another three reactions were seen in the treatment group, an acid taste and flatulence, hyperventilation, and neurological disturbances including a suspected grand mal. Three of the 20 controls also reported adverse reactions (May et al. 1996)." (BIBRA, 1999)

"No effect on growth was observed in six dogs given up to 125 mg/kg bw/day for 5 wk by capsule. Blood parameters were normal and a limited examination of the tissues (including the brain) failed to identity any treatment-related changes (Mengs & Stotzem, 1989)." (BIBRA, 1999)

"In nine studies, 269 healthy subjects or patients underwent exposure to peppermint oil (PO) either by topical intraluminal (stomach or colon) or oral administration by single doses or 2 weeks treatment (n = 19). Methods used to detect effects were oro-cecal transit time by hydrogen expiration, total gastrointestinal transit time by carmine red method, gastric emptying time by radiolabelled test meal or sonography, direct observation of colonic motility or indirect recording through pressure changes or relieve of colonic spasms during barium enema examination. The dose range covered in single dose studies is 0.1-0.24ml of PO/subject. With one exception, which show an unexplained potentiation of neostigmine stimulated colon activity, all other studies result in effects, indicating a substantial spasmolytic effect of PO of the smooth muscles of the gastrointestinal tract."

As taken from Grigoleit HG & GrigoletP. Phytomedicine. 2005 Aug; 12(8):607-11. PubMed, 2011 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=16121522&query_hl=19&itool=pubmed_DocSum

Record for CAS RN 8006-90-4:

Type of	Route of Exposure	Species / Test	Dose Data	Toxic Effects	Reference

Test	Exposure or Administration	/ Test System	Dose Data	Toxic Effects	Reference
TDLo - Lowest published toxic dose	Oral	Rodent - rat	9 gm/kg/90D (intermittent)	Brain and Coverings - other degenerative changes Kidney/Ureter/Bladder - changes in tubules (including acute renal failure, acute tubular necrosis)	TOLED5 Toxicology Letters. (Elsevier Science Pub. B.V., POB 211, 1000 AE Amsterdam, Netherlands) V.1- 1977- Volume(issue)/page/year: 62,215,1992
TDLo - Lowest published toxic dose	Oral	Rodent - rat	2.32 mL/kg/28D (intermittent)	Liver - other changes Biochemical - Enzyme inhibition, induction, or change in blood or tissue levels - phosphatases	CEXPB9 Clinical and Experimental Pharmacology and Physiology. (Blackwell Scientific Publications, (Australia) Pty Ltd., 107 Barry St., Carlton, Vic. 3053, Australia) V.1- 1974- Volume(issue)/page/year: 30,799,2003

As taken from RTECS, 2011

5.3. Reproduction toxicity

“Peppermint oil has been used to induce menstruation and should, therefore, be avoided in pregnancy.”

As taken from HSDB, 2003

Groups of 10 female rats were given oral doses of peppermint oil at 0, 150, 750 or 1500 mg/kg bw/day from 7 days prior to mating and throughout mating, gestation and delivery, until postnatal day 4. Maternal toxicity (statistically significant decreased body weight gain and food consumption and clinical effects) were seen at 750 mg/kg bw/day and above. A statistically significant increase in the number of stillborn pups was noted at 750 mg/kg bw/day and above, with decreased pup viability and pup body weight also being noted at these dose levels. The maternal and developmental NOAEL was 150 mg/kg/day (Vollmuth et al. 1990).

Mentha x piperita (CAS RN 8006-90-4) is suspected to be toxic for reproduction. The Toolbox profiler DART scheme v.1.0 gives an alert for toxicity to reproduction.

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.

5.4. Mutagenicity

The Ames test was used to evaluate the mutagenicity of a number of neat complex flavor mixtures. Studies in which peppermint oil was part of the test mixture include EMT000309 (CD-ROM 1, JTI Submission, 2002). The results show that these mixtures were not mutagenic.

Peppermint oil was not mutagenic when tested in a number of Salmonella strains in the presence and absence of metabolic activation (BIBRA, 1999). However, the maximum concentration tested in the assay was limited by the toxicity of the oil.

No mutagenicity was detected when peppermint oil was tested in an Ames test or a mouse lymphoma assay, both in the presence and absence of metabolic activation. Similarly, it did not induce unscheduled DNA synthesis in rat hepatocytes *in vitro* (Heck *et al*., 1989)

Peppermint produced equivocal results in chromosome aberration assays in Chinese hamster lung cells in the absence of S9 (BIBRA, 1999).

"Genotoxic properties of the essential oils extracted from dill (Anethum graveolens L.) herb and seeds, peppermint (Menthaxpiperita L.) herb and pine (Pinus sylvestris L.) needles were studied using chromosome aberration (CA) and sister chromatid exchange (SCE) tests in human lymphocytes in vitro, and Drosophila melanogaster somatic mutation and recombination test (SMART) in vivo. In the CA test, the most active essential oil was from dill seeds, then followed essential oils from dill herb, peppermint herb and pine needles, respectively. In the SCE test, the most active essential oils were from dill herb and seeds followed by essential oils from pine needles and peppermint herb. Essential oils from dill herb and seeds and pine needles induced CA and SCE in a clear dose-dependent manner, while peppermint essential oil induced SCE in a dose-independent manner. All essential oils were cytotoxic for human lymphocytes. In the SMART test, a dose-dependent increase in mutation frequency was observed for essential oils from pine and dill herb. Peppermint essential oil induced mutations in a dose-independent manner. Essential oil from dill seeds was almost inactive in the SMART test."

As taken from Lazutka JR. (2001) 39(5):485-92. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=11313115&query_hl=11&itool=pubmed_docsum

"A Japanese group has reported both equivocal (Ishidate et *al.* 1984) and negative (Ishidate et *al.* 1988) results in tests on peppermint oil for chromosomal damage (aberrations) in Chinese hamster lung cells in the absence of a liver metabolic activation system." (BIBRA, 1999)

In vivo				
Species	**Test conditions**	**Endpoint**	**Result**	**Reference**
Drosophila Melanogaster (this assay can be classed as in vivo or in vitro)	0.1-1.5% peppermint oil fed to larvae for roughly 48 hr, adults then examined for wing spots	Somatic mutation and recombination	Weak +ve	Lazutka et al. 2001
No other relevant data identified for peppermint oil, but the following information on menthol (which may be present at up to 60% in peppermint oil) is pertinent.				
Rats (groups of 5-15)	Gavage administration of lmenthol at up to 145 mg/kg bw/day for 1 or 5 days, bone marrow examined for aberrations	Chromosome damage	-ve	LBI, 1973
Rats	Up to 3 g l-menthol/kg bw (probably oral, not specified in expert report), bone marrow examined for aberrations	Chromosome damage	-ve	LBI, 1975, cited in JECFA, 1999
Rats	Up to 3 g l-	Germ cell	-ve	LBI, 1975,

	menthol/kg bw (probably oral, not specified in expert report), dominant lethal assay (i.e. presumably early foetal deaths monitored when males were mated with untreated females)	mutations		cited in JECFA, 1999
Mice (groups of 5-6 males)	dl-menthol, 0, 0.25, 0.5 or 1 g/kg bw/day for 3 days by i.p. injection, bone marrow cells assessed for micronuclei. Top dose killed 3/6 mice	Chromosome damage	-ve (This was a high quality study)	Shelby et al. 1993
Mice (4)	Gavage administration of dl-menthol at 3 g/kg bw, one mouse killed at each timepoint 0, 3, 8 or 24 hours, DNA damage assessed (comet assay) in stomach, colon, liver, kidney, bladder, lung, brain and bone marrow	DNA damage	-ve (This was a high quality study)	Sasaki et al. 2000

In vitro

Test system	Test conditions	Endpoint	Activation	Result	References
Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537	Peppermint oil at up to 160 µg/plate. Higher concentrations were said to be toxic	Mutation	With and without S9	-ve The low concentration tested would have limited sensitivity	Andersen & Jensen, 1984 (cited in BIBRA, 1999)
Salmonella typhimurium strains TA98 and TA102	10 mg extract from the peppermint plant, Mentha piperita L.	Mutation	Without	+ve in TA102	Mahmoud et al. 1992
Salmonella typhimurium strains TA98 and TA100	Paper in Japanese. (Retrieval in Toxline search	Mutation	Mutation With and without S9	-ve (as were all of the tested agents)	Haresaku et al. 1985

	indicates that peppermint oil was tested.)				
Salmonella typhimurium, strains TA98, TA1535, TA1537 and TA1538	Peppermint I and II essential oils were said to have been tested at 5 and 10 picolitres/plate	Mutation	Without	Peppermint II was said to be +ve in TA1537 peppermint I weakly +ve in TA98 and TA1538. However, the study is unreliable as these positive results were reported at minuscule levels (picolitres/ plate) and spontaneous mutation rate was around 100-fold higher than normal	Sivaswamy et al. 1991
Bacillus subtilis	Peppermint oil tested at 5 µl/disc	DNA repair	?	+ve	Anon, 1985, (cited in RTECS, 2011)
Bacillus subtilis (rec assay)	Peppermint oil tested, conditions not specified in expert review	DNA repair	With and without S9	+ve in absence of S9	Kuroda et al. 1989 (cited in BIBRA, 1999)
Chinese hamster lung cells	Peppermint oil tested at up to 0.25 mg/ml, cells examined for chromosomal aberrations at 24 hr	Chromosome damage	Without	Equivocal (7% of cells had structural aberrations)	Ishidate et al. 1984
Chinese hamster lung cells	Peppermint oil tested for chromosomal aberrations. No further details given in expert review	Chromosome damage	Without	-ve	Ishidate et al. 1988 (cited in BIBRA, 1999)
Mouse lymphoma cells (L5178Y TK+/-)	Peppermint oil. No further details given in expert review.	Mutation	Without	-ve	Heck et al. 1989 (cited in BIBRA, 1999)

Rat liver cells	Peppermint oil. Cells assessed for sister chromatid exchanges (SCEs). No further details given in expert review.	Chromosome effects	?	-ve	Heck et al. 1989 (cited in BIBRA, 1999)
Human lymphocytes	Peppermint oil tested at up to 0.3 µl/ml, cells assessed for chromosome aberrations and SCEs	Chromosome damage and chromosome effects	Without	+ve for chromosome damage, equivocal for SCEs	Lazutka et al. 2001
No other relevant data identified for peppermint oil, but the following information on menthol (which may be present at up to 60% in peppermint oil) is pertinent.					
Salmonella typhimurium strains TA92, TA94, TA98, TA100, TA1535, TA1537, TA2637, G46	Various studies on dland l-menthol, at up to 5 mg/plate	Mutation	With and without S9	-ve	JECFA, 1999 (citing 4 studies)
Hamster ovary and lung cells, and human embryo cells	Various studies on dland l-menthol, at up to 10 mg/ml, cells assessed for chromosome aberrations and SCE	Chromosome damage and chromosome effects	With and without S9	-ve	JECFA, 1999 (citing 6 studies)
Hamster ovary cells	Incubated with dl-menthol at up to 1.8 mmol/l for 3 hr, harvested at 20 hr and assessed for chromosome aberrations. Toxic at 1.2 mmol/l and above	Chromosome damage	With and without S9	-ve at nontoxic concentrations, weak +ve at toxic concentrations	Hilliard et al. 1998
Mouse lymphoma cells	Two studies on dl-menthol, up to 0.2 mg/ml	Somatic cell mutations	Not specified in expert report	-ve	JECFA, 1999 (citing 2 studies)
Human blood cells and hamster lung	Up to 2 mmol/l, cells examined for	DNA damage	Not specified in expert	-ve	JECFA, 1999 (citing 1

cells	DNA damage		report		study)
Rat hepatocytes	Up to 1.3 mmol/l, cells examined for DNA damage	DNA damage	Not applicable	+ve	+ve JECFA, 1999 (citing 1 study)
+ve, positive; -ve, negative; ?, equivocal; with, with metabolic activation; without, without metabolic activation					

5.5. Cytotoxicity

“We have investigated whether peppermint oil inhibits heat shock-induced apoptosis of astrocytes. We found that peppermint oil inhibits the heat shock-induced apoptosis in both human astrocyte CCF-STTG1 cells and rat astrocytes. Pretreatment of the cells with peppermint oil inhibited the heat shock-induced DNA fragmentation and condensation of nuclear chromatin. Peppermint oil also inhibited the caspase-3 activation and poly-ADP-ribose polymerase fragmentation in CCF-STTG1 cells. These results suggest that peppermint oil may modulate the apoptosis of astrocytes via the activation of the caspase-3.”

As taken from Koo HN et al., (2001) J Mol Neurosci. 2001 Dec; 17(3):391-6. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=11859935&query_hl=19&itool=pubmed_DocSum

“Peppermint oils have been reported to have cytotoxic properties.”As taken from Encyclopaedia of common natural ingredients used in food, drugs and cosmetics, 2nd edition, A. Leung & S. Foster, 2003, pp. 368-372.

“Mentha piperita (MP), also known as peppermint, is an aromatic and medicinal plant widely used in the food industry, perfumery and cosmetic, pharmacy and traditional medicine. Its essential oil (EO) displays antimicrobial activity against a range of bacteria and fungi. In this study, we found that MP EO lethal cytotoxicity is associated with increased levels of intracellular reactive oxygen species, mitochondrial fragmentation and chromatin condensation, without loss of the plasma membrane integrity, indicative of an apoptotic process. Overexpression of cytosolic catalase and superoxide dismutases reverted the lethal effects of the EO and of its major component menthol. Conversely, deficiency in Sod1p (cytosolic copper-zinc-superoxide dismutase) greatly increased sensitivity to both agents, but deficiency in Sod2p (mitochondrial manganese superoxide dismutase) only induced sensitivity under respiratory growth conditions. Mentha piperita EO increased the frequency of respiratory deficient mutants indicative of damage to the mitochondrial genome, although increase in mitochondrial thiol oxidation does not seem to be involved in the EO toxicity.” As taken from Ferreira P et al. 2014. FEMS Yeast Res. 14(7), 1006-1014. PubMed, available at http://www.ncbi.nlm.nih.gov/pubmed/25065265

“The essential oil was obtained by hydrodistillation and the identification and quantification of components were achieved with the use of GC-MS analysis. The antioxidant activity was evaluated by the method of sequestration of DPPH. Essential oils were used for study the cytotoxic front larvae of Artemia salina. In the evaluation of the antimicrobial activity of essential oils, we employed the disk-diffusion method. The potential larvicide in mosquito larvae of the third stage of development of Aedes aegypti to different concentrations of essential oils was evaluated. The major compounds found in the essential oils of M. piperita were linalool (51.8%) and epoxyocimene (19.3%). The percentage of antioxidant activity was 79.9 ± 1.6%. The essential oil showed LC50 = 414.6 µg/mL front of A. saline and is considered highly toxic. It shows sensitivity and halos significant inhibition against E. coli. The essential possessed partial larvicidal efficiency against A. aegypti.” As taken from da Silva Ramos R et al. 2017. Scientific World Journal 2017, 4927214. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28116346

“The inhibitory potential by contact and vapor of basil, cinnamon, clove, peppermint, oregano, rosemary, common thyme, and red thyme essential oils (EOs) against 20 strains of Streptococcus suis was determined by the disk diffusion test. The broth microdilution method was used to determine the minimal inhibitory and minimal bactericidal concentration (MIC and MBC) of the four selected oils. Furthermore, the bactericidal power (ratio MBC/MIC) was calculated. The EOs with the major potential in the disk diffusion method were red thyme, common thyme, oregano, and cinnamon (Ø mean 16.5-34.2 mm), whereas cinnamon did not show vapor activity. In the microdilution test, all the EOs showed notable antimicrobial activity (MIC_{90} and MBC_{90} 312.5-625 $\mu g \cdot ml^{-1}$) and a strong bactericidal power (ratio = 1). This is the first study that selects essential oils against S. suis. New studies about the possible synergic effect of EOs with antibiotics and about toxicity and efficacy in in vivo conditions are recommended.” As taken from de Aguiar FC et al. 2018. Microbiologyopen. Epub ahead of print. PubMed, 2018 available at: https://www.ncbi.nlm.nih.gov/pubmed/29575822

“The present study aimed to investigate the phytochemical composition of *Mentha* × *piperita* L. (MP) and *Lavandula angustifolia* Mill. (LA) extracts in terms of hydroxycinnamic acid (HCAs) content, in particular, caffeic (CA), p-cumaric (CU), ferulic (FE), and rosmarinic (RS) acids using LC-MS. Also, the *in vitro* antimicrobial effect against *Staphylococcus aureus* and the antiproliferative activity against two cancerous cell lines (A375 and MDA-MB-231) using the MTT assay were tested. The extracts were prepared using aromatic water which resulted from the extraction of oils from plants as extraction medium, with/without acid. The results showed that RS and FE represent the majority of HCAs compounds; the highest content of FE is found in LA (7.47 $mg \cdot g^{-1}$d.m.), and the maximum content of RS in MP (6.36 $mg \cdot g^{-1}$d.m.). Regarding the antimicrobial effect against *Staphylococcus aureus*, the two extracts showed a simulative role on the growth rate of *Staphyloccocus aureus*, but a slightly inhibitory effect (69.12%) can be attributed to the acidic environment. In terms of biological activity against MDA-MB-231 breast carcinoma cell line, and A375 human melanoma cell line, at the highest employed concentration, 150 $\mu g \cdot mL^{-1}$, the tested extracts present a weak antiproliferative effect.” As taken from Alexa A et al. 2018. Anal. Cell Pathol. (Amst). 2018, 2678924. PubMed, 2018 available at: https://www.ncbi.nlm.nih.gov/pubmed/29552454

“In the present study, the essential oils (EOs) of some officinal plants from Abruzzo territory (Italy) were evaluated for their antimicrobial and antioxidant activities and their volatile fraction chemical characterization. The EOs were extracted from *Rosmarinus officinalis*, *Origanum vulgare*, *Salvia officinalis*, *Mentha piperita*, *Allium sativum*, *Foeniculum vulgare*, *Satureja montana*, *Thymus vulgaris* and *Coriandrum sativum* seeds. The antimicrobial activity was screened against thirteen Gram-positive and Gram-negative strains to determine the Minimal Inhibitory Concentration (MIC). The total phenolic content (TPC) and the antioxidant capacity (AOC) were assessed by means of Folin-Ciocâlteu method, and Trolox Equivalent Antioxidant Capacity with 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (TEAC/ABTS), Ferric Reducing Antioxidant Power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays respectively. Among the nine EOs tested, *T. vulgaris*, *S. montana*, *O. vulgare* and *C. sativum* EOs showed MIC values ranging from 0.625 to 5 µL/mL. The AOC and TPC results for these species were also interesting. The major components for these EOs were thymol for *T. vulgaris* (44%) and *O. vulgare* (40%), linalool (77%) for *C. sativum*, and carvacrol for *S. montana* (54%). The results allowed the study to establish that these EOs are good candidates for potential application as biopreservatives in foods and/or food manufacture environments.” As taken from Pellegrini M et al. 2018. Foods 7(2), E19. PubMed, 2018 available at: https://www.ncbi.nlm.nih.gov/pubmed/29393893

“This study was planned in order to investigate effective essential oils to inhibit in-vitro growth of Methicillin resistant Staphylococcus aureus (MRSA). In this study using disc diffusion method anti MRSA activity of ten diverse essential oils extracted from traditional plants namely Thymus vulgaris L, Mentha pulegium, Ocimum sanctum, Mentha piperita, Cymbopogon citratus, Rosmarinus officinalis L., Cortex cinnamom, Citrus nobilis x Citrus deliciosa, Origanum vulgare and Mentha sp. was examined. All the essential oils inhibited growth of S. aureus to different extent, by exhibiting moderate to elevated zones of inhibitions. Essential oils of cinnamon (Cortex cinnamomi) and thyme (Thymus vulgaris L) were observed to be the most powerful against MRSA strains used in

this study. At lowest concentration of 25µl/ml essential oils comprehensible zone of inhibition was found 9±0.085mm and 8±0.051mm respectively, and at elevated concentrations there was a total decline in growth of MRSA and a very clear zone of inhibition was observed. A synergistic effect of essential oils in amalgamation with amoxicillin a Penicillin group of antibiotic was also examined. Interestingly a strong synergism was observed with oregano (Origanum vulgare) and pennyroyal mint (Mentha pulegium) essential oils, which were not so effective alone driven out to be important synergistic candidate. Our results demonstrated that essential oils of cinnamon and thyme can be used as potential antimicrobial agent against the Methicillin-resistant Staphylococcus aureus infections and Amoxicillin antibacterial activity can be enhanced using active constituents present in oregano and pennyroyal mint essential oils." As taken from Uzair B et al. 2017. Pak. J. Pharm. Sci. 30(5(Supplementary)), 1997-2002. PubMed, 2018 available at: https://www.ncbi.nlm.nih.gov/pubmed/29105634

"Mentha piperita L. (peppermint) possesses antimicrobial properties, but little is known of its ability to modulate macrophages. Macrophages are essential in bacterial infection control due to their antimicrobial functions and ability to link the innate and adaptive immune responses. We evaluated the effects of the peppermint leaf hydroalcoholic extract (LHAE) on cultured murine peritoneal macrophages stimulated or not with lipopolysaccharide (LPS) in vitro. Vehicle-treated cells were used as controls. The constituents of the extract were also identified. Epicatechin was the major compound detected in the LHAE. LPS-induced macrophage death was reversed by incubation with LHAE (1-30 µg/ml). Higher concentrations of the extract (≥100 µg/ml) decreased macrophage viability (49-57%) in the absence of LPS. LHAE (1-300 µg/ml) attenuated H_2O_2 (34.6-53.4%) but not nitric oxide production by these cells. At similar concentrations, the extract increased the activity of superoxide dismutase (15.3-63.5-fold) and glutathione peroxidase (34.4-73.6-fold) in LPS-treated macrophages. Only LPS-unstimulated macrophages presented enhanced phagocytosis (3.6-6.6-fold increase) when incubated with LHAE (3-30 µg/ml). Overall, the LHAE obtained from peppermint modulates macrophage-mediated inflammatory responses, by stimulating the antioxidant pathway in these cells. These effects may be beneficial when the excessive activation of macrophages contributes to tissue damage during infectious disease." As taken from Arruda MO et al. 2017. J. Immunol. Res. 2017, 2078794. PubMed, 2018 available at: https://www.ncbi.nlm.nih.gov/pubmed/29085843

5.6. Carcinogenicity

Species	Test conditions	Evidence of carcinogenicity	Reference
Groups of 52 male mice	Oral administration (by stomach tube) of toothpaste providing 4 or 16 mg peppermint oil/kg bw/day, 6 days/wk for 80 wks. The incidence of tumours was compared with that in 260 male mice given the toothpaste base alone.	None No evidence of carcinogenic activity was seen in the brain, liver, lung or kidney, and the incidence of malignant lymphoma was similarly unaffected. This study was not designed to examine the carcinogenic potential of peppermint oil and would have had only a very limited sensitivity to this particular component.	Roe et al. 1979
No other relevant data identified for peppermint oil, but the following information on menthol (which may be present at			

up to 60% in peppermint oil) is pertinent.

Mice	Skin application of condensed vapour from mentholated cigarettes, results compared with mice treated with condensate from non-menthol cigarettes, no further details given in this very brief German report	None Tumour yields were similar in the two groups	Schievelbein, 1969
Groups of 50 rats/sex	Dietary inclusion of dl-menthol to give doses of about 190 or 375 mg/kg bw/day for 2 yr. A comprehensive examination of tissues was undertaken	None	NCI, 1978
Groups of 50 mice/sex	Dietary inclusion of dl-menthol to give doses of about 300 or 600 mg/kg bw/day for 2 yr. A comprehensive examination of tissues was undertaken	No real evidence Small increases in liver carcinoma in males – 8/48, 8/48, 14/48 – and benign lung tumours in females – 0/49, 3/47, 5/48 – were not statistically significant and were within historical control ranges	NCI, 1978

5.7. Irritation/immunotoxicity

Peppermint oil is not generally considered as an irritant of human skin, but it does cause allergic contact dermatitis (BIBRA, 1999). Peppermint oil has been shown to be irritant on exposure with rabbit skin.

The use of peppermint oil in toothpastes, mouth washes and foods has resulted in sensitization reactions in and around the mouth, and a asthmatic responses have also been reported (BIBRA, 1999).

“Splash of peppermint oil in the eye caused a loss of corneal epithelium, corneal infiltration, release of pigment into the anterior chamber with deposits on the back of the cornea, but in the course of sixteen days the irritation subsided.”

As taken from HSDB, 2003

“We report 12 cases of contact sensitivity to the flavouring agents menthol and peppermint oil in patients presenting with intra-oral symptoms in association with burning mouth syndrome, recurrent oral ulceration or a lichenoid reaction. The patients were referred from the Glasgow Dental Hospital over a 4-year period for assessment of the possible contribution of contact sensitivity to their complaints. 5 patients with burning mouth syndrome demonstrated contact sensitivity to menthol and/or peppermint, with 1 patient sensitive to both agents, 3 positive to menthol only and 1 to peppermint only. 4 cases with recurrent intra-oral ulceration were sensitive to both menthol and peppermint. 3 patients with an oral lichenoid reaction were positive to menthol on patch testing, with 2 also sensitive to peppermint. 9 of the 12 cases demonstrated additional positive patch test results. After a mean follow-up of 32.7 months (range 9-48 months), of the 9 patients that could be contacted, 6 patients described clearance or improvement of their symptoms as a consequence of avoidance of menthol/peppermint.”

As taken from Morton CA et al. (1995) Contact Dermatitis. 1995 May; 32(5):281-4. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=7634781&query_hl=13&itool=pubmed_docsum

“Peppermint oil can produce-urticarial reactions [hives] (de Groot, 1994).” (BIBRA, 1999)

“The etiology of cheilitis is often not readily apparent. We present a case series of four patients with allergic contact cheilitis (ACC) secondary to exposure to peppermint oil contained in a lip balm product. These patients developed eczematous dermatitis involving their lips and perioral skin. They were tested with the North American Contact Dermatitis Group standard series as well as with an expanded series of flavoring agents, sunscreens, plant and fragrance components, and their own products. The lip balm contained potential sensitizers such as propolis, lanolin, coconut oil, almond oil, peppermint oil, and vitamin E. Our patch-test results showed that peppermint oil was the most likely culprit in these patients' ACC. Peppermint oil is less commonly reported as causing ACC than are more common contactants such as balsam of Peru or nickel sulfate. However, with the widespread use of lip balms containing peppermint oil, more cases of peppermint oil-induced ACC may be expected” (Tran et al. 2010).

In a review of patch test results in a large cohort of patients, doubtful or irritant reactions to peppermint oil were more frequent than positive reactions (Uter et al 2010).

Peppermint absolute (Mentha piperita) is considered to be an established contact allergen in humans (SCCS, 2011).

Vulval contact dermatitis was diagnosed in a 43-year-old woman who regularly drank large quantities of peppermint oil-containing herbal tea over a prolonged period. She also tested positive to a patch test with 2% peppermint oil (Vermatt et al. 2008).

A case of allergic contact dermatitis to the peppermint fragrance in foot spray as a secondary event to golfer’s vasculitis has been reported in a 57-year-old woman. The patient tested positive in a patch test to the diluted fragrance (Kalavala et al. 2007).

“BACKGROUND: Anaphylaxis, a form of IgE mediated hypersensitivity, arises when mast cells and possibly basophils are provoked to secrete mediators with potent vasoactive and smooth muscle contractile activities that evoke a systemic response. We report a case of IgE mediated anaphylaxis to peppermint (Mentha piperita) in a male shortly after sucking on a candy. CASE PRESENTATION: A 69 year old male developed sudden onset of lip and tongue swelling, throat tightness and shortness of breath within five minutes of sucking on a peppermint candy. He denied lightheadedness, weakness, nausea, vomiting, or urticaria. He took 25 mg of diphenhydramine, but his symptoms progressed to onset of cough, wheeze and difficulty with talking and swallowing. He was rushed to the nearest emergency department, where he was treated with intramuscular epinephrine, antihistamines and steroids. On history, he reported recent onset of mouth itchiness and mild tongue and lip swelling after using Colgate peppermint toothpaste. He denied previous history of asthma, allergic rhinitis, food or drug allergies. His past medical history was remarkable for hypercholesterolemia, gastroesophageal reflux and gout. He was on simvastatin, omeprazole, aspirin, and was carrying a self-injectable epinephrine device. He moved to current residence three years ago and cultivated mint plants in his backyard. He admitted to develop nasal congestion, cough and wheeze when gardening. Physical examination was unremarkable apart from slightly swollen pale inferior turbinates. Skin prick test (SPT) was strongly positive to a slurry of peppermint candy and fresh peppermint leaf, with appropriate controls. Same tests performed on five healthy volunteers yielded negative results. Skin testing to common inhalants including molds and main allergenic foods was positive to dust mites. Strict avoidance of mint containing items was advised. Upon reassessment, he had removed mint plants from his garden which led to resolution of symptoms when gardening. CONCLUSION: IgE mediated anaphylaxis to peppermint is rare. This case demonstrates a systemic reaction to a commonly consumed item, incapable of triggering anaphylaxis in the far majority of the population, yet causing a severe episode for our patient.” As taken from Bayat R and Borici-Mazi R. 2014. Allergy Asthma Clin. Immunol. 10(1), 6. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24472564

“Topically applied cosmetics and medicaments containing botanical extracts are commonly used. Despite popular beliefs of their benignancy, some botanicals have been implicated in causing

allergic contact dermatitis in susceptible patients. The offending allergen may be the botanical extract itself or another ingredient such as a fragrance, preservative, dye, or sunscreen found in the product. Specific botanicals implicated in causing cosmetic contact dermatitis include Compositae family plants, tea tree oil, peppermint, lavender, lichens, henna, and others." As taken from Jack AR et al. 2013. Semin. Cutan. Med. Surg. 32(3), 140-6. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24175401

"Peppermint oil is easily available as a constituent of medicines. A near fatal case due to ingestion of toxic dose of oral peppermint oil is being reported. The patient came in a comatosed state and was in shock. She was managed with mechanical ventilation and ionotropes. Her vital parameters reached normal within 8 hours and became conscious by 24 hours. The side effects of peppermint oil are considered to be mild but this case report warns that ingestion of oral toxic doses of peppermint oil could be dangerous." As taken from Nath SS et al. 2012. Indian J. Anaesth. 256(6), 582-4. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23325948

"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. pallescens (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 intraperitonealy and by the hot plate test in mice. The results showed a menthol chemotype for M. piperita pallescens, 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

5.8. All other relevant types of toxicity

No inhalation data were identified for peppermint oil, but the following information on menthol (which may be present at up to 60% in peppermint oil) is pertinent.

Damage to the liver and kidneys, as well as irritation of the eyes and respiratory tract occurred in workers at a factory producing menthol and a range of fragrance oils. Menthol levels in the air ranged between 12-380 mg/m3 (Kowalski et al. 1962). A 13-yr-old child who inhaled an estimated 4 mg menthol/kg bw in a volatile oil preparation showed euphoria, involuntary eye movement, double vision and unsteadiness, that lasted for less than 12 hr (O'Mullane et al. 1982). A woman developed digestive disorders, vomiting, irritability, unsteadiness, insomnia, speech disorders, tremors, confusion, mental depression and slowed heart rate after smoking 80 mentholated cigarettes daily for 3 months (Luke, 1962). The symptoms disappeared three weeks after she changed to a non-mentholated brand. One mentholated cigarette contains up to 4 mg menthol, and taking absorption data into account, apparently provides a dose of about 0.01 mg/kg bw (Schievelbein, 1969).

Damage to the nasal passages and sinuses was reported in rabbits which inhaled an aerosol of a 1% solution of menthol once daily for 9 months [dose unspecified] (Fox, 1930). No overt toxicity or damage to the respiratory system occurred when groups of four rats inhaled up to approximately 0.9 mg l-menthol/m3, 6.75 hr/day, 5 days/wk for a maximum of 52 exposures. At about 1.6 mg/m3 the blood picture was normal, and detailed examination of a range of tissues revealed only effects on the lung, nearly all rats showing congestion and inflammation (Rakieten et al. 1954). "Regression changes" in the liver and kidney of mice apparently occurred after inhalation of air containing 100 mg/m3 for 3 months [exposure regime unspecified] (Kowalski et al. 1962). No overt

toxicity was reported when four monkeys inhaled, for 8 hr/day, an aerosol providing about 40 mg menthol/kg bw/day for 14 days (Alarie, 1976).

“This study was undertaken to determine the influences of various doses of peppermint oil on the hepatic en-zymes, alanine transaminase, apartate tranaminase, alkaline phosphotase and gamma glutamyl transferase and the level of malondialdehyde in the serum of mice with and without immobility stress. The mice exposed to drink water, 0.9, 27 and 60 mg/kg peppermint oil from the days 1 to 5 for a period of 4 h before and after immobility stress. Serum MDA in-creased in treatment group II, III and IV after immobility stress. There was a significant decrease in ALT in treatment group III and IV after immobility stress. There were also significant decreases in ALP and GGT in treatment group IV af-ter immobility stress. This result may suggest that, MDA level is higher in immobilization stress group than in the un-immobilized animals in serum and this results show that enzyme activities decreased after immobilization stress.” As taken from Marjani A et al. 2012. Open Biochem. J. 6, 51-5. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22654997

“The present study aimed to evaluate the antimicrobial activity of peppermint oil against Staphylococcus aureus, and further investigate the influence of peppermint oil on S. aureus virulence-related exoprotein production. The data show that peppermint oil, which contained high contents of menthone, isomenthone, neomenthol, menthol, and menthyl acetate, was active against S. aureus with minimal inhibitory concentrations (MICs) ranging from 64-256 μg/mL, and the production of S. aureus exotoxins was decreased by subinhibitory concentrations of peppermint oil in a dose-dependent manner. The findings suggest that peppermint oil may potentially be used to aid in the treatment of S. aureus infections.” As taken from Li J et al. 2011. Molecules 16(2), 1642-54. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/21326141?dopt=AbstractPlus

“…….Time-kill assay was performed to compare the microbicidal activity of Olbas and peppermint oil during several time intervals. Olbas displayed a high antimicrobial activity against all test strains used in this study, among them antibiotic resistant MRSA (methicillin-resistant Staphylococcus aureus) and VRE (vancomycin-resistant Enterococcus). Its antimicrobial activity was comparable to that of peppermint oil which was the most potent one of all individual essential oils tested. In the time kill assay Olbas as well as peppermint oil demonstrated similar microbicidal activities…….” As taken from Hamoud R et al. 2012. Phytomedicine 19(11), 969-76. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22739414

"This overview of systematic reviews (SRs) aims to evaluate critically the evidence regarding the adverse effects of herbal medicines (HMs). Five electronic databases were searched to identify all relevant SRs, with 50 SRs of 50 different HMs meeting our inclusion criteria. Most had only minor weaknesses in methods. Serious adverse effects were noted only for four HMs: Herbae pulvis standardisatus, Larrea tridentate, Piper methysticum and Cassia senna. The most severe adverse effects were liver or kidney damage, colon perforation, carcinoma, coma and death. Moderately severe adverse effects were noted for 15 HMs: Pelargonium sidoides, Perna canaliculus, Aloe vera, Mentha piperita, Medicago sativa, Cimicifuga racemosa, Caulophyllum thalictroides, Serenoa repens, Taraxacum officinale, Camellia sinensis, Commifora mukul, Hoodia gordonii, Viscum album, Trifolium pratense and Stevia rebaudiana. Minor adverse effects were noted for 31 HMs: Thymus vulgaris, Lavandula angustifolia Miller, Boswellia serrata, Calendula officinalis, Harpagophytum procumbens, Panax ginseng, Vitex agnus-castus, Crataegus spp., Cinnamomum spp., Petasites hybridus, Agave americana, Hypericum perforatum, Echinacea spp., Silybum marianum, Capsicum spp., Genus phyllanthus, Ginkgo biloba, Valeriana officinalis, Hippocastanaceae, Melissa officinalis, Trigonella foenum-graecum, Lagerstroemia speciosa, Cnicus benedictus, Salvia hispanica, Vaccinium myrtillus, Mentha spicata, Rosmarinus officinalis, Crocus sativus, Gymnema sylvestre, Morinda citrifolia and Curcuma longa. Most of the HMs evaluated in SRs were associated with only moderately severe or minor adverse effects. As taken from Posadzki P et al. 2013. Clin. Med. 13(1), 7-12. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23472485

“We present natural polymeric composite films made of essential oils (EOs) dispersed in sodium alginate (NaAlg) matrix, with remarkable anti-microbial and anti-fungal properties. Namely, elicriso

italic, chamomile blue, cinnamon, lavender, tea tree, peppermint, eucalyptus, lemongrass and lemon oils were encapsulated in the films as potential active substances. Glycerol was used to induce plasticity and surfactants were added to improve the dispersion of EOs in the NaAlg matrix. The topography, chemical composition, mechanical properties, and humidity resistance of the films are presented analytically. Antimicrobial tests were conducted on films containing different percentages of EOs against Escherichia coli bacteria and Candida albicans fungi, and the films were characterized as effective or not. Such diverse types of essential oil-fortified alginate films can find many applications mainly as disposable wound dressings but also in food packaging, medical device protection and disinfection, and indoor air quality improvement materials, to name a few." As taken from Liakos I et al. 2013. Int. J. Pharm. 463(2), 137-45. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24211443

"CONTEXT: Gastrointestinal disorders are common childhood complaints. Particular types of complementary and alternative medicine, such as herbal medicine, are commonly used among children. Research information on efficacy, safety, or dosage forms is still lacking. OBJECTIVES: To systematically summarize effectiveness and safety of different herbal treatment options for gastrointestinal disorders in children. DATA SOURCES: Medline/PubMed, Scopus, and the Cochrane Library were searched through July 15, 2016. STUDY SELECTION: Randomized controlled trials comparing herbal therapy with no treatment, placebo, or any pharmaceutical medication in children and adolescents (aged 0-18 years) with gastrointestinal disorders were eligible. DATA EXTRACTION: Two authors extracted data on study design, patients, interventions, control interventions, results, adverse events, and risk of bias. RESULTS: Fourteen trials with 1927 participants suffering from different acute and functional gastrointestinal disorders were included in this review. Promising evidence for effectiveness was found for Potentilla erecta, carob bean juice, and an herbal compound preparation including Matricaria chamomilla in treating diarrhea. Moreover, evidence was found for peppermint oil in decreasing duration, frequency, and severity of pain in children suffering from undifferentiated functional abdominal pain. Furthermore, evidence for effectiveness was found for different fennel preparations (eg, oil, tea, herbal compound) in treating children with infantile colic. No serious adverse events were reported. LIMITATIONS: Few studies on specific indications, single herbs, or herbal preparations could be identified. CONCLUSIONS: Because of the limited number of studies, results have to be interpreted carefully. To underpin evidence outlined in this review, more rigorous clinical trials are needed.." As taken from Anheyer D et al. 2017. Pediatrics 139(6), e20170062. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28562281

"Peppermint leaves are widely used for the symptomatic treatment of digestive disorders. Previous studies have shown significant effects of its natural products on human enzyme activity; however, there is no study available concerning the effects of peppermint tea on metabolizing enzymes in humans. Aim of the present study was to investigate the effect of peppermint tea on CYP1A2, CYP2A6, Xanthine Oxidase (XO), N-acetyltranferase-2 (NAT2) and UDP-glucuronosyltransferases-1A1/1A6 (UGT1A1/1A6) activities in healthy subjects. Four males and five females consumed peppermint tea (2 g of dry leaves/200 mL water, twice daily) for six days. CYP1A2, CYP2A6, XO, NAT2 and UGT1A1/1A6 activities were determined before and at the end of the study period, using the following caffeine and paracetamol metabolic ratios: CYP1A2: 17MX/137MX (saliva) and (AFMU+1MU+1MX)/17MU (urine); CYP2A6: 17MU/(17MU + 17MX), XO: 1MU/(1MU+1MX), NAT2, AFMU/(AFMU+1MU+1MX) and UGT1A1/1A6 glucuronidated/total paracetamol, all determined in urine. NAT2 metabolic ratio was significantly reduced following peppermint consumption (0.15 ± 0.13 vs 0.14 ± 0.13; $p < 0.05$). CYP1A2 urine and saliva indices were reduced, yet not significantly, following peppermint consumption (urine: 3.17 ± 1.08 vs 2.91 ± 0.76, saliva: 0.56 ± 0.12 vs 0.50 ± 0.12; $p > 0.05$). Peppermint had no influence on CYP2A6, XO and UGT1A1/1A6 indices. Daily ingestion of peppermint tea may alter pharmacokinetics of clinically administered drugs and promote cancer chemoprevention through NAT2 inhibition." As taken from Begas E et al. 2017. Food Chem. Toxicol. 100, 80-89. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28011360

"This study examined the effects of peppermint essential oil (PEP) on aerobic capacity. Seven healthy participants performed a graded maximal exercise test following 10 days of ingesting either

PEP or a control in a randomised crossover design. There was no significant difference between control and PEP trials for expired gas variables (peak oxygen uptake, 3.54 vs. 3.52 L/min) or performance measures (time to exhaustion, 583.33 vs. 587.04 s). Similarly, resting cardiopulmonary measures were also unchanged between visits." As taken from Shepherd K and Peart DJ. 2017. Appl. Physiol. Nutr. Metab. 42(5), 558-561. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28177705

"The inhibitory potential by contact and vapor of basil, cinnamon, clove, peppermint, oregano, rosemary, common thyme, and red thyme essential oils (EOs) against 20 strains of Streptococcus suis was determined by the disk diffusion test. The broth microdilution method was used to determine the minimal inhibitory and minimal bactericidal concentration (MIC and MBC) of the four selected oils. Furthermore, the bactericidal power (ratio MBC/MIC) was calculated. The EOs with the major potential in the disk diffusion method were red thyme, common thyme, oregano, and cinnamon (Ø mean 16.5-34.2 mm), whereas cinnamon did not show vapor activity. In the microdilution test, all the EOs showed notable antimicrobial activity (MIC_{90} and MBC_{90} 312.5-625 $\mu g \cdot ml^{-1}$) and a strong bactericidal power (ratio = 1). This is the first study that selects essential oils against S. suis. New studies about the possible synergic effect of EOs with antibiotics and about toxicity and efficacy in in vivo conditions are recommended." As taken from de Aguiar FC et al. 2018. Microbiologyopen. Epub ahead of print. PubMed, 2018 available at: https://www.ncbi.nlm.nih.gov/pubmed/29575822

6. Functional effects on

6.1. Broncho/pulmonary system

"We describe a case of acute lung injury following IV injection of peppermint oil. An 18-yr-old woman injected the oil and developed fulminant pulmonary edema requiring ventilator support. Within 4 h after injection her arterial oxygen tension was 8.1 kPa (60 mm Hg) at an inspired oxygen fraction (F(IO2)) of 0.7 (P/F ratio: 85) despite a positive end expiratory pressure (PEEP) of 20 mbar, therefore meeting criteria for acute respiratory distress syndrome (ARDS). Mean pulmonary artery pressures and pulmonary artery wedge pressures were within normal limits throughout the case (<25 mm Hg and <10 mm Hg, respectively). Ventilation with high PEEP and diuresis resulted in a P/F ratio of 265 after 24 h. The patient was successfully weaned from the ventilator on the 9th day. This report is the first description of the sequelae of IV peppermint oil injection. The injection resulted in pulmonary edema and acute lung injury, presumably due to direct toxicity and a resultant increase in pulmonary vascular permeability. This report is the first description of IV peppermint oil injection. The patient rapidly developed severe fluid overload of the lung and subsequent lung injury that required intubation, mechanical ventilation, and intensive care therapy for 13 days. The pulmonary edema was presumably caused by direct toxicity and an increase in pulmonary vascular permeability."

As taken from Behrends M et al., (2005), Anesth Analg. 2005 Oct; 101(4):1160-2. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=16192538&query_hl=9&itool=pubmed_docsum

"A Spaniard with a history of asthma suffered an attack after the ingestion of a menthol-flavoured sweet, and experienced an immediate reduction of lung function when brushing his teeth with similarly flavoured toothpaste. A 30-second mouth rinse with peppermint oil (8 mm3 diluted in 2 ml alcohol) produced an adverse reaction on lung function, as did a similar exposure to spearmint oil and menthol (Subiza *et al.* 1992). Wheezing from the chewing of gum containing "peppermint" has been reported in a US patient (Spurlock & Dailey, 1990)." (BIBRA, 1999)

"BACKGROUND: Enhancing athletic performance is a great desire among the athletes, coaches

and researchers. Mint is one of the most famous natural herbs used for its analgesic, anti-inflammatory, antispasmodic, antioxidant, and vasoconstrictor effects. Even though inhaling mint aroma in athletes has been investigated, there were no significant effects on the exercise performance. METHODS: Twelve healthy male students every day consumed one 500 ml bottle of mineral water, containing 0.05 ml peppermint essential oil for ten days. Blood pressure, heart rate, and spirometry parameters including forced vital capacity (FVC), peak expiratory flow rate (PEF), and peak inspiratory flow (PIF) were determined one day before, and after the supplementation period. Participants underwent a treadmill-based exercise test with metabolic gas analysis and ventilation measurement using the Bruce protocol. RESULTS: The FVC (4.57 ± 0.90 vs. 4.79 ± 0.84; $p < 0.001$), PEF (8.50 ± 0.94 vs. 8.87 ± 0.92; $p < 0.01$), and PIF (5.71 ± 1.16 vs. 6.58 ±1.08; $p < 0.005$) significantly changed after ten days of supplementation. Exercise performance evaluated by time to exhaustion (664.5 ± 114.2 vs. 830.2 ± 129.8 s), work (78.34 ±32.84 vs. 118.7 ± 47.38 KJ), and power (114.3 ± 24.24 vs. 139.4 ± 27.80 KW) significantly increased ($p < 0.001$). In addition, the results of respiratory gas analysis exhibited significant differences in VO2 (2.74 ± 0.40 vs. 3.03 ± 0.351 L/min; $p < 0.001$), and VCO2 (3.08 ± 0.47 vs. 3.73 ± 0.518 L/min; $p < 0.001$). CONCLUSIONS: The results of the experiment support the effectiveness of peppermint essential oil on the exercise performance, gas analysis, spirometry parameters, blood pressure, and respiratory rate in the young male students. Relaxation of bronchial smooth muscles, increase in the ventilation and brain oxygen concentration, and decrease in the blood lactate level are the most plausible explanations." As taken from Meamarbashi A and Rajabi A. 2013. J. Int. Soc. Sports Nutr. 10(1), 15. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23517650

6.2. Cardiovascular system

"Two human patients who chronically consumed large quantities of peppermint candy presented with idiopathic atrial fibrillation resistant to quinidine therapy."

"Idiopathic atrial fibrillation has occurred following chronic exposure.""Bradycardia has been reported."

As taken from HSDB, 2003

"This study examined the effects of peppermint essential oil (PEP) on aerobic capacity. Seven healthy participants performed a graded maximal exercise test following 10 days of ingesting either PEP or a control in a randomised crossover design. There was no significant difference between control and PEP trials for expired gas variables (peak oxygen uptake, 3.54 vs. 3.52 L/min) or performance measures (time to exhaustion, 583.33 vs. 587.04 s). Similarly, resting cardiopulmonary measures were also unchanged between visits." As taken from Shepherd K and Peart DJ. 2017. Appl. Physiol. Nutr. Metab. 42(5), 558-561. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28177705

6.3. Nervous system

"The cyst-like spaces in cerebellar tissue were not seen in five-week studies in which rats of the same strain were given peppermint oil at doses of 150 or 500 mg/kg bw per day and dogs were given peppermint oil in gelatin capsules daily at a dose of 25 or 125 mg/kg bw per day (Mengs & Stotzem, 1989)."

As taken from WHO Food Additives Series 42, available at http://www.inchem.org/documents/jecfa/jecmono/v042je21.htm

"The same encephalopathy was observed in rats administered 40 or 100 mg/kg bw/day peppermint oil, but not 10 mg/kg bw/day, for 28 days."

As taken from NTP, 2011, available at https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr563.pdf

“Postherpetic neuralgia remains a difficult problem to treat. A number of therapies have been shown to be effective, but some patients have intractable pain. The case of a 76-year-old woman whose pain had been resistant to standard therapies is described. The pattern of quantitative sensory testing results for this patient led the authors to believe that she had an "irritable nociceptor" type of pathophysiology. The patient was instructed to apply neat peppermint oil (containing 10% menthol) to her skin, resulting in an almost immediate improvement in her pain. This pain relief persisted for 4-6 hours after application of the oil. The patient was successfully treated with topical peppermint oil. During 2 months of follow-up she has had only a minor side effect, with continuing analgesia. The authors believe this is the first evidence of peppermint oil (or menthol) having a strong analgesic effect on neuropathic pain. The possible mechanisms of action of peppermint oil are discussed.”

As taken from Davies SJ et al., (2002), Clin J Pain. 2002 May-Jun; 18(3):200-2. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=12048423&query_hl=19&itool=pubmed_DocSum

“I investigated whether dopamine (DA) is involved in the ambulation promoted by pulegone (PUL), a constituent of peppermint oil, in ICR mouse. Co-administration of PUL and bupropion (BUP) had an additive effect on their ambulation-promoting activities. When administered with PUL, the DA antagonists chlorpromazine, fluphenazine, haloperidol, SCH12679, and spiperone all attenuated the effect of PUL on ambulation. In addition, pretreatment with the DA depletor reserpine produced no subsequent sensitivity to the effect of PUL. Taken together, DA may be involved in the ability of PUL to promote ambulation in ICR mice but PUL may not be a direct DA agonist. The chemical structure of PUL is similar to menthol and menthone, and thus they may all be acting through a common mechanism.”

As taken from Umezu T. Pharmacol Biochem Behav. 2010, Feb; 94(4):497-502. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=retrieve&db=pubmed&list_uids=19917306&dopt=AbstractPlus

“.....Our present study aimed to characterize and semi-quantify the radiation-induced apoptosis in CNS and the activity of Mentha extracts as neuron-protective agent. Our results through flow cytometry exhibited the significant disturbance and arrest in cell cycle in % of M1: SubG1 phase, M2: G0/1 phase of diploid cycle, M3: S phase and M4: G2/M phase of cell cycle in brain tissue ($p < 0.05$). Significant increase in % of apoptosis and P53 protein expression as apoptotic biomarkers were coincided with significant decrease in Bcl(2) as an anti-apoptotic marker. The biochemical analysis recorded a significant decrease in the levels of reduced glutathione, superoxide dismutase, deoxyribonucleic acid (DNA) and ribonucleic acid contents. Moreover, numerous histopathological alterations were detected in brain tissues of gamma irradiated mice such as signs of chromatolysis in pyramidal cells of cortex, nuclear vacuolation, numerous apoptotic cell, and neural degeneration. On the other hand, gamma irradiated mice pretreated with Mentha extract showed largely an improvement in all the above tested parameters through a homeostatic state for the content of brain apoptosis and stabilization of DNA cycle with a distinct improvement in cell cycle analysis and antioxidant defense system. Furthermore, the aforementioned effects of Mentha extracts through down-regulation of P53 expression and up-regulation of Bcl(2) domain protected brain structure from extensive damage. Therefore, Mentha extract seems to have a significant role to ameliorate the neuronal injury induced by gamma irradiation.” As taken from Hassan HA et al. 2013. Cytotechnology 65(1), 145-56. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/23011739

“BACKGROUND: Mentha piperita L. (Labiatae) is an herbaceous plant, used in folk medicine for the treatment of several medical disorders. METHODS AND RESULTS: In the present study, the aqueous extract of Mentha piperita leaf, at the i.p doses 200 and 400 mg/kg, showed significant analgesic effects against both acetic acid-induced writhing and hot plate-induced thermal stimulation in mice, with protection values of 51.79% and 20.21% respectively. On the contrary,

the Mentha piperita leaf aqueous extract did not exhibit anti-inflammatory activity against carrageenan induced paw oedema. CONCLUSION: These findings indicate that Mentha piperita has a potential analgesic effect that may possibly have mediated centrally and peripherally, as well as providing a pharmacological evidence for its traditional use as a pain reliever." As taken from Taher YA. 2012. Libyan J. Med. 2012, 7. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22468149

"Although plant-derived essential oils (EOs) have been used to treat various mental disorders, their central nervous system (CNS) acting effects have not been clarified. The present study compared the effects of 20 kinds of EOs with the effects of already-known CNS acting drugs to examine whether the EOs exhibited CNS stimulant-like effects, CNS depressant-like effects, or neither. All agents were tested using a discrete shuttle-type conditioned avoidance task in mice. Essential oils of peppermint and chamomile exhibited CNS stimulant-like effects; that is, they increased the response rate (number of shuttlings/min) of the avoidance response....." As taken from Umezu T. 2012. Phytother. Res. 26(6), 884-91. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22086772

"Attachment to an abusive caregiver has wide phylogenetic representation, suggesting that animal models are useful in understanding the neural basis underlying this phenomenon and subsequent behavioral outcomes. We previously developed a rat model, in which we use classical conditioning to parallel learning processes evoked during secure attachment (odor-stroke, with stroke mimicking tactile stimulation from the caregiver) or attachment despite adversity (odor-shock, with shock mimicking maltreatment). Here we extend this model to mice. We conditioned infant mice (postnatal day (PN) 7-9 or 13-14) with presentations of peppermint odor and either stroking or shock. We used (14) C 2-deoxyglucose (2-DG) to assess olfactory bulb and amygdala metabolic changes following learning. PN7-9 mice learned to prefer an odor following either odor-stroke or shock conditioning, whereas odor-shock conditioning at PN13-14 resulted in aversion/fear learning. 2-DG data indicated enhanced bulbar activity in PN7-9 preference learning, whereas significant amygdala activity was present following aversion learning at PN13-14. Overall, the mouse results parallel behavioral and neural results in the rat model of attachment, and provide the foundation for the use of transgenic and knockout models to assess the impact of both genetic (biological vulnerabilities) and environmental factors (abusive) on attachment-related behaviors and behavioral development." As taken from Roth TL et al. 2013. Genes Brain Behav. 12(7), 673-80. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23927771

"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

"OBJECTIVES: The objective of this pilot study was to determine the effectiveness of a mixture of

essential oils (peppermint, basil, and helichrysum) on mental exhaustion, or moderate burnout (ME/MB) using a personal inhaler. DESIGN: This was a randomized, controlled, double-blind pilot study. Data were collected 3 times a day for 3 weeks (Monday-Friday). The first week was baseline for both groups, the second week was intervention (aromatherapy or placebo), and the third week was washout. SETTINGS/LOCATION: Participants used a personal inhaler at home or at work. Subjects: The subjects comprised a convenience sample of 13 women and 1 man who each had self-assessed ME/MB. INTERVENTIONS: Participants were randomized to receive a personal inhaler containing either a mixture of essential oils or rose water (as used in Indian cooking). OUTCOME MEASURES: The outcome measures were a 0-10 scale with 10=worst feeling of burnout, 0=no feeling of burnout. There was a qualitative questionnaire rating aroma and a questionnaire listing perceived stressors. RESULTS: While both groups had a reduction in perception of ME/MB, the aromatherapy group had a much greater reduction. CONCLUSIONS: The results suggest that inhaling essential oils may reduce the perceived level of mental fatigue/burnout. Further research is warranted." As taken from Varney E and Buckle J. 2013. J. Altern. Complement. Med. 19(1), 69-71. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23140115

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

It has been claimed that the direct instillation of pepermint oil into the colon can cause both muscle relaxation and stimulation (BIBRA, 1999).

"Peppermint extracts have been reported to have antiviral activities against Newcastle disease, herpes simplex, vaccinia, Semliki Forest, and West Nile viruses in egg and cell-culture system. Peppermint oil has been demonstrated to exhibit spasmolytic activity on smooth muscles of experimental animals." As taken from Encyclopaedia of common natural ingredients used in food, drugs and cosmetics, 2nd edition, A. Leung & S. Foster, 2003, pp. 368-372

"In a double blind crossover trial, the use of 0.2 mL of oral peppermint oil capsule, 3-6 capsules/day, in the tratement of irritable bowel syndrome was studied in 29 patients given peppermint oil or placebo. Results showed that treated patients felt significantly better with less abdominal symptoms, stool frequency and side effects."

As taken from Dew MJ et al., (1984), Br J Clin Pract. 1984 Nov-Dec; 38(11-12):394, 398. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=6397219&query_hl=1&itool=pubmed_DocSum

"Peppermint oil was moderately potent reversible inhibitor of in vitro CYP3A4 activity. Grapefruit juice increased the oral bioavailability of felodipine by inhibition of CYP3A4-mediated presystemic drug metabolism. Peppermint oil may also have acted by this mechanism. However, this requires further investigation. Ascorbyl palmitate did not inhibit CYP3A4 activity in vivo."

As taken from Dresser GK et al., (2002), Clin Pharmacol Ther. 2002 Sep; 72(3):247-55. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=12235445&query_hl=5&itool=pubmed_docsum

"Systemic administration of a cholinergic blocking agent or glucagon is used to reduce spasms, but it is inconvenient and sometimes causes side effects. This study is an evaluation of the intracolonic administration of peppermint oil during colonoscopy for the control of colonic spasm. Each patient in the treated group (n = 409) was given approximately 200 mL of the solution (a mixture of 8 mL of peppermint oil and 0.2 mL of Tween 80 per 1 L of water with 0.04% indigo carmine) by using a hand pump attached to the accessory channel of the colonoscope. Changes in patient posture were made to distribute the solution. The patients in the control group (n = 36) were given the solution without peppermint oil. A satisfactory spasmolytic effect was seen in 88.5% of the treated patients and in 33.3% of those in the control group ($p<0.0001$). No adverse effect was observed. The mean time to onset was 21.6 +/- 15.0 seconds, and the effect continued for at least 20 minutes. In

patients with irritable bowel syndrome, efficacy was significantly lower ($p < 0.0001$). The intraluminal administration of peppermint oil by using a hand pump is a simple, safe, and convenient alternative to the systemic injection of a cholinergic blocking agent or glucagon during colonoscopy."

As taken from Asao T et al., (2001), Gastrointest Endosc. 2001 Feb; 53(2):172-7. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=11174287&query_hl=7&itool=pubmed_docsum

"GI endoscopy without general anesthesia causes a hyperperistaltic state in the stomach, which frequently necessitates the use of antispasmodic agents, such as hyoscine-N-butylbromide, but these drugs have side effects. Peppermint oil is harmless and acts locally to inhibit GI smooth muscle contraction. A randomized double-blind, double-dummy, controlled trial was conducted in 100 patients to compare the antispasmodic effects of hyoscine-N-butylbromide administered intramuscularly and a placebo solution administered intraluminally by means of the endoscope, and also the effects of a placebo solution administered intramuscularly with those of a peppermint oil solution administered intraluminally. The percent change in diameter of the pyloric ring before and after the administrations was defined as the opening ratio, and the percent change in diameter between the maximally and minimally opened pyloric ring states was defined as the contraction ratio. Time until disappearance of the contraction ring(s) in the gastric antrum and side effects of the drugs were also determined. The opening ratio was significantly higher in the peppermint oil administration group than in the hyoscine-N-butylbromide injection group. The contraction ratio after peppermint oil administration was significantly lower than that after hyoscine-N-butylbromide injection. The time required for disappearance of the antral contraction ring(s) was shorter in the peppermint oil group (97.1 +/- 11.4) than in the hyoscine-N-butylbromide group (185.9 +/- 10.1 s; $p < 0.0001$). No significant side effects were associated with peppermint oil, whereas hyoscine-N-butylbromide injection produced side effects such as dry mouth, blurred vision, and urinary retention. Peppermint oil solution administered intraluminally can be used as an antispasmodic agent with superior efficacy and fewer side effects than hyoscine-N-butylbromide administered by intramuscular injection during upper endoscopy."

As taken from Hiki N et al., (2003), Gastrointest Endosc. 2003 Apr; 57(4):475-82. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=12665756&query_hl=19&itool=pubmed_DocSum

"OBJECTIVES: Intestinal peristalsis can impede explorations and interventions using retrograde endoscopic cholangiopancreatography. Systemic spasmolytics are frequently employed to reduce this phenomenon, in spite of the adverse anti-cholinergic effects they are associated with. We proposed a formula using 1.6% peppermint oil solution with local use in order to avoid these adverse side effects. METHOD: We formulated a preparation of 1.6% peppermint oil solution in accordance with the medical literature. The effectiveness of the formula was evaluated in a semi-qualitative manner according to the reduction in peristalsis. RESULTS: We tested two different emulgents, and polysorbate provided the best results. The pilot study carried out with 8 patients demonstrated its effectiveness and safety in reducing intestinal peristalsis. CONCLUSIONS: 1.6% peppermint oil solution constitutes an effective and safe alternative to the use of systemic spasmolytics....." As taken from Solà-Bonada N et al. 2012. Farm. Hosp. 36 (4), 256-60. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22137159?dopt=AbstractPlus

"BACKGROUND: Menthol reduces intestinal motility in animal studies, an effect that is probably mediated by transient receptor potential channels. Peppermint oil (PO), with menthol as a major constituent, is widely used as a spasmolytic agent in irritable bowel syndrome. In the current study, we investigated the effect of acute PO administration on intragastric pressure (IGP) profiles and gastric sensorimotor functions in health. METHODS: Healthy volunteers underwent IGP measurement before and during continuous intragastric infusion of a nutrient drink (n = 13), and gastric barostat studies (n = 13). A single capsule of PO (182 mg) or placebo was administered during the studies in a randomized controlled crossover design. Throughout the studies, healthy

volunteers scored 11 epigastric symptoms on a visual analogue scale (VAS); satiation was scored on a 6-point Likert scale during intragastric infusion. KEY RESULTS: During fasting, IGP and motility index (MI) of the proximal stomach decreased significantly after PO administration compared with placebo ($P < 0.0001$ and <0.05, respectively). In contrast, during intragastric infusion of the nutrient drink, no significant differences were detected between PO and placebo in IGP profiles, MI, satiation scores, and epigastric symptoms. The maximum infused volume, gastric compliance or sensitivity to balloon distention did not differ between both treatment arms. However, reduced appetite scores were seen during fasting after PO treatment, as compared with placebo ($P = 0.01$). Postprandial VAS scores were similar between PO and placebo. CONCLUSIONS & INFERENCES: Peppermint oil reduces IGP, proximal phasic contractility, and appetite, with negligible effects on gastric sensitivity, tone, accommodation, and nutrient tolerance in health." As taken from Papathanasopoulos A et al. 2013. Neurogastroenterol. Motil. 25(4), e263-71. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/23489975

"Irritable bowel syndrome (IBS) is one of the most common functional gastrointestinal disorder which is associated with considerable sufferings of patient and Peppermint oil is volatile oil, its active principle is menthol-contain a cyclic monoterpine which has anti-spasmotic properties due to its ability to block calcium channel of intestinal smooth muscles. This study observed the efficacy of peppermint oil for relieving the symptoms and changes of quality of life (QOL) in diarrhea predominant IBS. This was a prospective double blind randomized placebo-controlled study conducted in the Bangabandhu Sheikh Mujib Medical University during July 2008 to September 2009. Patients who fulfilled ROME II were initially selected but those had red flag signs or any organic disease was excluded from the study. Seventy four patients were enrolled in the study and randomly allocated to receive either peppermint oil or placebo three times daily for six weeks. Changes of symptoms were assessed three week interval during treatment and two weeks after the end of treatment. Data were analyzed by paired and unpaired 't' test. Finally sixty five patients completed the trial. It was observed that, at six weeks of therapy abdominal pain is markedly improved (mean±SD) 4.94±1.30 in peppermint oil group compared with 6.15±1.24 in placebo group and the difference was statistically highly significant ($p>0.001$). But two weeks after end of trials pain score again increased (6.09±1.93). Other symptoms and quality of life did not improve significantly. So the study result concludes that peppermint oil is effective in reliving only abdominal pain in diarrhea predominant IBS transiently." As taken from Alam MS et al. 2013. Mymensingh Med. J. 22(1), 27-30. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/23416804

"Irritable bowel syndrome (IBS) is the most prevalent functional disease of the gastrointestinal tract. This highly prevalent condition is best diagnosed by assessing the constellation of symptoms with which patients present to their physicians. Because some critics have previously questioned whether irritable bowel syndrome and other functional gastrointestinal disorders truly exist because they do not have defining structural features, the Rome Foundation fostered the use of symptom-based criteria for universal use. In most cases treatment is reduced to symptomatic therapy because a lot of unknown in pathogenesis by irritable bowel syndrome. Irritable bowel syndrome leads to decrease of quality of life of the patients and could be one of the reasons of patients' disability. Food is believed by patients promotes symptoms and the diet or avoiding specific food can reduce symptoms. Possible role of different food and microbiota in the pathophysiology of irritable bowel syndrome, as well as the data from randomized, controlled clinical trials dedicated to the effects of diet in irritable bowel syndrome are summarized and discussed in this review. The efficacy of the diet, enriched by fiber, prebiotics, probiotics, peppermint oil, curcumin and vitamin B6 in irritable bowel syndrome patients was shown in numerous studies. In some studies restriction in consumption of fermented carbohydrates, coffee and alcohol, as well as diet with elimination IgG-sensed food was also shown to be effective in irritable bowel syndrome. Food intolerances, defined as non-toxic non-immune adverse reactions to food, include reactions to bioactive chemicals in foods and metabolic reactions to poorly absorbed dietary carbohydrates. New dietary approaches like polyunsaturated fatty acids intake correction and the low tryptophan intake are discussed." As taken from Pilipenko VI et al. 2013. Vopr. Pitan. 82(1), 64-73. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23808281?dopt=AbstractPlus

"The diagnosis of irritable bowel syndrome (IBS) should be considered when patients have had

abdominal pain/discomfort, bloating, and change in bowel habits for 6 months. Patients may experience variation between periods of constipation and diarrhea. When evaluating patients with IBS, physicians should be alert for red flag symptoms, such as rectal bleeding, anemia, nighttime pain, and weight loss. Physicians also should consider other medical conditions that manifest similarly to IBS. Clinicians who are confident in diagnosing IBS based on symptoms typically do not obtain many tests unless the patient has red flag symptoms. Various etiologic mechanisms have been proposed for IBS, including abnormal bowel motility, inflammation, altered mucosal permeability, genetic predisposition, and visceral hypersensitivity. Lack of certainty about the etiology makes it difficult to develop effective management approaches; thus, management is directed toward symptom relief. Dietary changes, such as avoiding fermentable carbohydrates, may benefit some patients, especially those with bloating. Constipation-dominant IBS can be managed with antispasmodics, lubiprostone, or linaclotide, whereas diarrhea-dominant IBS can be managed with loperamide or alosetron, though the latter drug can cause ischemic colitis. For long-term therapy, tricyclic antidepressants or selective serotonin reuptake inhibitors have good efficacy. Peppermint oil and probiotics also may provide benefit." As taken from Fashner J and Gitu AC. 2013. Family Physician Essentials 413, 16-23. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24124703

"GOALS: The aim of this study was to assess the efficacy and safety of enteric-coated peppermint oil capsules compared with placebo for the treatment of active irritable bowel syndrome (IBS). BACKGROUND: IBS is a common disorder that is often encountered in clinical practice. Medical interventions are limited and the focus is on symptom control. STUDY: Randomized placebo-controlled trials with a minimum treatment duration of 2 weeks were considered for inclusion. Cross-over studies that provided outcome data before the first cross-over were included. A literature search upto February 2013 identified all applicable randomized-controlled trials. Study quality was evaluated using the Cochrane risk of bias tool. Outcomes included global improvement of IBS symptoms, improvement in abdominal pain, and adverse events. Outcomes were analyzed using an intention-to-treat approach. RESULTS: Nine studies that evaluated 726 patients were identified. The risk of bias was low for most of the factors assessed. Peppermint oil was found to be significantly superior to placebo for global improvement of IBS symptoms (5 studies, 392 patients, relative risk 2.23; 95% confidence interval, 1.78-2.81) and improvement in abdominal pain (5 studies, 357 patients, relative risk 2.14; 95% confidence interval, 1.64-2.79). Although peppermint oil patients were significantly more likely to experience an adverse event, such events were mild and transient in nature. The most commonly reported adverse event was heartburn. CONCLUSIONS: Peppermint oil is a safe and effective short-term treatment for IBS. Future studies should assess the long-term efficacy and safety of peppermint oil and its efficacy relative to other IBS treatments including antidepressants and antispasmodic drugs." As taken from Khanna R et al. 2014. J. Clin. Gastroenterol. 48(6), 505-12. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24100754

"Functional abdominal pain (FAP) is a common complaint among children and adolescents. For many patients, symptoms exacerbate with eating. This review discusses findings concerning the role of diet in FAP. The foods that are discussed are divided into 2 major groups: food allergies or intolerances, which focus on milk, gluten, and fermentable oligosaccharides, disaccharides, monosaccharides, and polyols; and functional foods, which hone in on foods that reduce abdominal pain in adolescents such as fiber, peppermint oil, and probiotics. Lastly, we discuss the role of eating habits in FAP and how the physiology of eating may be the real culprit of symptoms associated with eating." As taken from van Tilburg and Felix CT. 2013. J. Pediatr. Gastroenterol. Nutr. 57(2), 141-8. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23698023

"PURPOSE: With little scientific evidence to support use of aromatherapy for postoperative nausea and/or vomiting (PONV) symptoms, this study evaluated controlled breathing with peppermint aromatherapy (AR) and controllled breathing alone (CB) for PONV relief. DESIGN: A single blind randomized control trial design was used. METHODS: On initial PONV complaint, symptomatic

subjects received either CB (n = 16) or AR (n = 26) intervention based on randomization at enrollment. A second treatment was repeated at 5 minutes if indicated. Final assessment occurred 10 minutes post initial treatment. Rescue medication was offered for persistent symptoms. FINDINGS: Among eligible subjects, PONV incidence was 21.4% (42/196). Gender was the only risk factor contributing to PONV symptoms (P = .0024). Though not statistically significant, CB was more efficacious than AR, 62.5% versus 57.7%, respectively.
CONCLUSIONS: CB can be initiated without delay as an alternative to prescribed antiemetics. Data also support use of peppermint AR in conjunction with CB for PONV relief." As taken from Sites DS et al. 2014. J. Perianesth. Nurs. 29(1), 12-9. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24461278

"BACKGROUND: Approximately 80 percent of pregnant women suffer by some degree of nausea and vomiting. But the treatment of nausea and vomiting of pregnancy is rarely successful. OBJECTIVES: The aim of this study was evaluation the effect of mint on nausea and vomiting during pregnancy that its treatment in some recent research has been effective. MATERIALS AND METHODS: In this double blind RCT, 60 pregnant women with nausea and vomiting of pregnancy were sampled and divided into two groups with Block-randomized method. mint group, in addition to giving the routine training, for four consecutive nights, before sleeping, a bowel of water whit four drops of pure mint essential oil placed on the floor near their beds and in control groups were used four drops of normal saline . The severity of nausea by using Visual Analog Scale (VAS) and severity of vomiting by counting the number of its in 7 days prior, 4 days during, and 7 days after intervention were assessed. RESULTS: The results showed that the severity of nausea and vomiting did not differ between the two groups in 7days before and after intervention by using repeated measurement test. But during intervention, the severity of nausea showed a decreasing trend (especially in 4th night) in the mint and an increasing trend in the control group. The severity of nausea within 7 days after the intervention had a decreasing trend in both groups; however, the intensity was lower in the mint than saline group but not statically significant. No meaningful relationship has been detected during and after intervention for the intensity of vomiting. CONCLUSIONS: The results of study showed that peppermint essential oil hasn't the effect on nausea and vomiting of pregnancy." As taken from Pasha H et al. 2012. Iran. Red Crescent Med. J. 14(11), 727-30. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23396673

"Itching is one of the most common skin symptoms. Generalized pruritus occurs in 1-8% of pregnant women. It can create unpleasant feeling for these women especially at nights. Most pregnant women avoid using synthetic drugs because of their side effects. Peppermint is a plant which has been used as a traditional drug in Iran. It decreases skin's temperature. This study was done to determine the effects of peppermint oil on symptomatic treatment of pruritus in pregnant women attending to Rasoul Akram Hospital in Rasht, 2011. In this triple-blind clinical trial, 96 randomly selected subjects diagnosed with pruritus gravidarum were studied (47 cases and 49 controls). A bottle containing 60 mL of peppermint oil 0.5% in sesame oil and identical placebos were provided to be taken twice a day during 2 weeks by the case and control groups, respectively. The severity of the itch was assessed and compared before and after the study by VAS system. The results were analyzed by SPSS. Statistical methods such as descriptive analysis, independent samples' t-test, paired samples' t-test and Chi-square were employed. The severity of the itch in the treated group with peppermint oil in comparison with the placebo group, showed a significant statistical difference (p = 0.003). In accordance with the results of this study, it seems that peppermint oil can be effective in reducing the severity of Pruritus Gravidarum. More studies with larger sample sizes are required to confidently declare the mentioned results." As taken from Akhavan Amjadi M et al. 2012. Iran. J. Pharm. Res. 11(4), 1073-7. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24250539

"OBJECTIVE: To report a migraineur with osmophobia and trigger to garlic and onion aroma. BACKGROUND: While odors serve as a trigger in 70% of migraineurs, alliaceous aromas have been described only rarely. Furthermore, nor has more than one type of alliaceous odor acted as a trigger in the same individual. Neither has migraine with aura been described as precipitated by

such aromas. A patient experiencing migraines with aura, triggered almost exclusively by alliaceous aromas, is described. METHODS: Case study: 32-year-old woman; 5 years previously felt nasal pruritis upon eating a red onion dip. Shortly thereafter, the mere aroma of raw onions caused a sensation of her throat closing along with an associated panic attack. Over the intervening years, upon exposure to onions and garlic aroma she experienced a fortification spectra and visual entopia, followed by a bipareital, crushing level 10/10 headache, burning eyes and nose, lacrimation, perioral paresthesias, generalized pruritis, nausea, fatigue, sore throat, dysarthria, confusion, dyspnea, palpitations, presyncopal sensations, hand spasms, tongue soreness, neck pain, phonophobia, and photophobia. These would persist for 1 hour after leaving the aroma. She was unresponsive to medication and would wear a surgical mask when out. The patient also experienced chemosensory complaints: dysosmias every few months; phantosmias of food or cleaning products every month for a minute of level 5/10 intensity; pallinosmia of onion or garlic odor for 30 minutes after exposure; and metallic pallinugeusia after eating with metal utensils. RESULTS: Neurological exam normal except for bilateral positive Hoffman reflexes. CHEMOSENSORY TESTING: Quick Smell Identification Test 3/3 and Brief Smell Identification Test 12/12 were normal. Magnetic resonance imaging and computed tomography with and without contrast normal. Allergy skin test was positive for garlic and onion. Nose plug and counter stimulation with peppermint prevented the onset of headaches and associated symptoms. CONCLUSION: This is the first report of migraines with aura triggered by more than one alliaceous compound in the same individual. Possible mechanisms include odor induced, emotional change, vasomotor instability, trigeminal-induced neurogenic inflammation, and allergic response. In alliaceous and odor-induced migraines, a trial of counter stimulation and nose plugs is warranted." As taken from Roussos AP and Hirsch AR. 2014. Headache 54(2), 378-82. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23551212

"BACKGROUND: Antibacterial treatments currently used for treatment cause several side effects, and bacterial resistance to the antibiotics is also increasing. Therefore, there is need to find better alternatives. Essential oils (EOs) have been used for treatment of various ailments since ancient times and have gained popularity over the years. Safety and efficacy of EOs have been proved by several clinical trials. This review gives an overview on the EOs, their uses, and adverse effects. MATERIALS AND METHODS: A literature search was performed in the PubMed for clinical trial studies and review articles on EOs published up to February 2015. The search was performed during March 2015. The following keywords were used: "Lavender essential oil," "cinnamon oil," "clove oil," "eucalyptus oil," "peppermint oil," "lemon EOs," and "tea tree oil." RESULTS: Total 70 relevant articles were found in PubMed database. After screening of abstracts, 52 articles were selected to be included in the present review. CONCLUSION: On the basis of the available information, it can be concluded that EOs have the potential to be developed as preventive or therapeutic agents for various oral diseases, but further clinical trials are required to establish their safety and efficacy." As taken from Dagli N et al. 2015. J. Int. Soc. Prev. Community Dent. 5(5), 335-40. PubMed, 2016 available at: http://www.ncbi.nlm.nih.gov/pubmed/26539382

"OBJECTIVE: To systematically review literature assessing efficacy and safety of pharmacologic treatments in children with abdominal pain-related functional gastrointestinal disorders (AP-FGIDs). STUDY DESIGN: MEDLINE and Cochrane Database were searched for systematic reviews and randomized controlled trials investigating efficacy and safety of pharmacologic agents in children aged 4-18 years with AP-FGIDs. Quality of evidence was assessed using Grades of Recommendation, Assessment, Development and Evaluation approach. RESULTS: We included 6 studies with 275 children (aged 4.5-18 years) evaluating antispasmodic, antidepressant, antireflux, antihistaminic, and laxative agents. Overall quality of evidence was very low. Compared with placebo, some evidence was found for peppermint oil in improving symptoms (OR 3.3 (95% CI 0.9-12.0) and for cyproheptadine in reducing pain frequency (relative risk [RR] 2.43, 95% CI 1.17-5.04) and pain intensity (RR 3.03, 95% CI 1.29-7.11). Compared with placebo, amitriptyline showed 15% improvement in overall quality of life score (P = .007) and famotidine only provides benefit in global symptom improvement (OR 11.0; 95% CI 1.6-75.5; P = .02). Polyethylene glycol with tegaserod significantly decreased pain intensity compared with polyethylene glycol only (RR 3.60, 95% CI 1.54-8.40). No serious adverse effects were reported. No studies were found concerning

antidiarrheal agents, antibiotics, pain medication, anti-emetics, or antimigraine agents. CONCLUSIONS: Because of the lack of high-quality, placebo-controlled trials of pharmacologic treatment for pediatric AP-FGIDs, there is no evidence to support routine use of any pharmacologic therapy. Peppermint oil, cyproheptadine, and famotidine might be potential interventions, but well-designed randomized controlled trials are needed." As taken from Korterink JJ et al. 2015. J. Pediatr. 166(2), 424-31. PubMed, 2016 available at: http://www.ncbi.nlm.nih.gov/pubmed/25449223

"Herb-induced liver injury (HILI) has recently attracted attention due to increasing reports of hepatotoxicity associated with use of phytotherapeutics. Here, we present data on HILI from the Berlin Case-Control Surveillance Study. The study was initiated in 2000 to investigate the serious toxicity of drugs including herbal medicines. Potential cases of liver injury were ascertained in more than 180 Departments of all 51 Berlin hospitals from October 2002 to December 2011. Drug or herb intake was assessed through a standardized face-to-face interview. Drug or herbal aetiology was assessed based on the updated Council for International Organizations of Medical Sciences scale. In ten of all 198 cases of hepatotoxicity included in the study, herbal aetiology was assessed as probable (once ayurvedic herb) or possible (Valeriana five times, Mentha piperita once, Pelargonium sidoides once, Hypericum perforatum once, Eucalyptus globulus once). Mean age was 56.4 ± 9.7 years, and the predominant pattern of liver injury was hepatocellular. No cases of acute liver failure or death were observed. This case series corroborates known risks for ayurvedic herbs, supports the suspected association between Valeriana use and liver injury, and indicates a hepatotoxic potential for herbs such as Pelargonium sidoides, Hypericum perforatum or Mentha piperita that were rarely associated with liver injury before. However, given that possible causality does not prove clinical significance, further studies in this field are needed." As taken from Douros A et al. 2016. Int. J. Mol. Sci. 17(1), E114. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26784183

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 Peppermint absolute 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 Peppermint absolute. Table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
	39	Carmines, 2002 & Rustemeier et al., 2002

Smoke chemistry	211 (oil)	Baker et al., 2004a
	250 (No CAS) 2600	JTI KB Study Report(s)
	67,200	Gaworski et al., 2011 & Coggins et al., 2011b
	6	Roemer et al., 2014
In vitro genotoxicity	39	Carmines, 2002 & Roemer et al., 2002
	211 (oil)	Baker et al., 2004c
	250	Renne et al., 2006
	250 (No CAS) 4000	JTI KB Study Report(s)
	600	fGLH Study Report (2010)
	67,200	Gaworski et al., 2011 & Coggins et al., 2011b
	6	Roemer et al., 2014
In vitro cytotoxicity	39	Carmines, 2002 & Roemer et al., 2002
	211 (oil)	Baker et al., 2004c
	4000	JTI KB Study Report(s)
	600	fGLH Study Report (2010)
	67,200	Gaworski et al., 2011 & Coggins et al., 2011b

	6	Roemer et al., 2014
Inhalation study	2	Gaworski et al., 1998
	39	Carmines, 2002 & Vanscheeuwijck et al., 2002
	211 (oil)	Baker et al., 2004c
	250	Renne et al., 2006
	250 (No CAS) 4000	JTI KB Study Report(s)
	67,200	Gaworski et al., 2011 & Coggins et al., 2011b
	6	Schramke et al., 2014
Skin painting	66	Gaworski et al., 1999
	250 (No CAS)	JTI KB Study Report(s)
In vivo genotoxicity	6	Schramke et al., 2014

9. Heated/vapor emissions toxicity

No data available to us at this time.

10. Ecotoxicity

10.1. Environmental fate

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that oils, peppermint (CAS RN 8006-90-4) are not persistent in the environment.

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

EPISuite provides the following data:

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

Bond Method :	1.52E-005 atm-m3/mole (1.54E+000 Pa-m3/mole)
Group Method:	2.56E-005 atm-m3/mole (2.60E+000 Pa-m3/mole)
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:	HLC: 3.630E-006 atm-m3/mole (3.678E-001 Pa-m3/mole) VP: 0.00767 mm Hg (source: MPBPVP) WS: 435 mg/L (source: WSKOWWIN)

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

Log Kow used:	3.19 (exp database)
Log Kaw used:	-3.207 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate):	6.397
Log Koa (experimental database):	None

Probability of Rapid Biodegradation (BIOWIN v4.10):

Biowin1 (Linear Model):	0.8319
Biowin2 (Non-Linear Model) :	0.8707
Biowin3 (Ultimate Survey Model):	3.0138 (weeks)
Biowin4 (Primary Survey Model) :	3.7517 (days)
Biowin5 (MITI Linear Model) :	0.4554
Biowin6 (MITI Non-Linear Model):	0.3314
Biowin7 (Anaerobic Linear Model):	0.3226
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):	29.3 Pa (0.22 mm Hg)
Log Koa (Koawin est):	6.397
Kp (particle/gas partition coef. (m3/ug)): Mackay model: Octanol/air (Koa) model:	 1.02E-007 6.12E-007

Fraction sorbed to airborne particulates (phi):

Junge-Pankow model:	3.69E-006
Mackay model:	8.18E-006
Octanol/air (Koa) model:	4.9E-005

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

Hydroxyl Radicals Reaction:

OVERALL OH Rate Constant =	24.0849 E-12 cm3/molecule-sec
Half-Life =	0.444 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life =	5.329 Hrs
Ozone Reaction:	No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi):	5.94E-006 (Junge-Pankow, Mackay avg) 4.9E-005 (Koa method)
Note: the sorbed fraction may be resistant to atmospheric oxidation	

Soil Adsorption Coefficient (KOCWIN v2.00):

Koc :	88.15 L/kg (MCI method)
Log Koc:	1.945 (MCI method)
Koc :	189.7 L/kg (Kow method)
Log Koc:	2.278 (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: 2.56E-005 atm-m3/mole (estimated by Group SAR Method)

Half-Life from Model River:	29.87 hours (1.244 days)
Half-Life from Model Lake:	430.6 hours (17.94 days)

Removal In Wastewater Treatment:

Total removal:	8.92 percent
Total biodegradation:	0.14 percent
Total sludge adsorption:	7.48 percent
Total to Air:	1.30 percent

(using 10000 hr Bio P,A,S)

Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	1.2	10.7	1000
Water	27.8	360	1000
Soil	70.9	720	1000
Sediment	0.15	3.24e+003	0

Persistence Time: 417 hr

10.2. Aquatic toxicity

The Ecological Categorization Results from the Canadian Domestic Substances List state that oils, peppermint (CAS RN 8006-90-4) are not inherently toxic to aquatic organisms:

Pivotal value for iT (mg/l)	22.3
Comment iT	Group: individual; Subgroup: Other oils (individual);
Toxicity to fathead minnow (LC50 in mg/l) as predicted by Topkat v6.1	22.3
Toxicity to fish (LC50 in mg/l) as predicted by Ecosar v0.99g	5.844
Toxicity to fish (LC50 in mg/l) as predicted by Oasis Forecast M v1.10	32.899
Toxicity to fish (LC50 in mg/l) as predicted by Aster	8.907547

Toxicity to fish (LC50 in mg/l) as predicted by PNN	38.16727
Toxicity to fish, daphnia, algae or mysid shrimp (EC50 or LC50 in mg/l) as predicted by Ecosar v0.99g	0.629
Chronic toxicity to daphnia or algae (EC50 in mg/l) as predicted by Ecosar v0.99g	0.646
Toxicity to fish (LC50 in mg/l) as predicted by Neutral Organics QSAR in Ecosar v0.99g	5.84E+000

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

ECOSAR version 1.11 reports the following aquatic toxicity data for CAS RN 8006-90-4:

Values used to Generate ECOSAR Profile
Log Kow: 3.381 (EPISuite Kowwin v1.68 Estimate)
Wat Sol: 490 (mg/L, PhysProp DB exp value)

ECOSAR v1.11 Class-specific Estimations
Neutral Organics

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Neutral Organics :	Fish	96-hr	LC50	7.379
Neutral Organics :	Daphnid	48-hr	LC50	4.760
Neutral Organics :	Green Algae	96-hr	EC50	6.008
Neutral Organics :	Fish		ChV	0.838
Neutral Organics :	Daphnid		ChV	0.662
Neutral Organics :	Green Algae		ChV	2.090
Neutral Organics :	Fish (SW)	96-hr	LC50	9.367
Neutral Organics :	Mysid	96-hr	LC50	2.731
Neutral Organics :	Fish (SW)		ChV	2.363
Neutral Organics :	Mysid (SW)		ChV	0.157

“This study was aimed to assess the potential effects of Mentha piperita on the hemato - immunological and biochemical parameters, skin antibacterial activity and protection against Yersinia ruckeri infection in rainbow trout Oncorhynchus mykiss. Fish were divided into 4 groups before being fed diets supplemented with 0, 1, 2 and 3% of Mentha piperita (MP) plant extract for 8 weeks. Dose-dependent increases immune (both in skin mucus and blood serum) and hematological parameters (number of red and white cells, hematocrit and hemoglobin contents), as well as in respiratory burst activity, total protein, albumin, and neutrophil levels in fish fed

supplemented diets compared to the control fish. Furthermore, dietary MP plant extract supplements have no significant effect on blood biochemical parameters and enzymatic activities of liver determined in serum of rainbow trout. After 8 weeks the cessation of feeding with MP plant extract, survival rates of 54.4%, 63.6% and 75.2% were recorded in groups which received 1, 2 and 3% of MP plant extract of feed, respectively, compared to 34.6% survivals in the control. This study underlying several positive effects of dietary administration of MP plant extract to farmed fish." As taken from Adel M et al. 2016. Fish Shellfish Immunol. 55, 267-73. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27245867

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

"The oviposition deterrence and ovicidal potential of five different essential oils, peppermint oil (Mentha piperita), basil oil (Ocimum basilicum), rosemary oil (Rosemarinus officinalis), citronella oil (Cymbopogon nardus), and celery seed oil (Apium graveolens), were assessed against female adults of the dengue vector, Aedes aegypti L. Multiple concentration tests were carried out where cups containing 1 mL of different concentrations (100%, 10%, 1%, 0.1%) of the oils and 199 mL of water were used for oviposition. The number of eggs laid and the larvae hatched in each cup were scored to evaluate the oviposition deterrent and ovicidal potentials of the oils. Our investigations revealed that the addition of 100% oil (pure oil) caused complete oviposition deterrence except in A. graveolens which resulted in 75% effective repellency. The use of 10% oil resulted in the maximum deterrence of 97.5% as shown by the M. piperita oil while other oils caused 36-97% oviposition deterrence as against the control. The oviposition medium with 1% oil showed decreased deterrent potential with 30-64% effective repellency, the M. piperita oil being exceptional. However, as the concentrations of the oil were reduced further to 0.1%, the least effective oil observed was A. graveolens (25% ER). Also, the M. piperita oil showed much reduced activity (40%) as compared to the control, while the other oils exhibited 51-58% repellency to oviposition. The studies on the ovicidal effects of these oils revealed that the eggs laid in the water with 100% essential oils did not hatch at all, whereas when 10% oils were used, only the R. officinalis oil resulted in 28% egg hatch. At lower concentrations (1%), the oils of M. piperita, O. basilicum, and C. nardus showed complete egg mortality while those of A. graveolens and R. officinalis resulted in 71% and 34% egg hatches, respectively. When used at 0.1%, the O. basilicum oil was found to be the only effective oil with 100% egg mortality, whereas other oils resulted in 16-76% egg mortality, the least mortality caused by the A. graveolens oil. These results suggest that these essential oils can be employed in a resistance-management program against A. aegypti. Further detailed research is needed to identify the active ingredient in the extracts and implement the effective mosquito management program." As taken from Saharkhiz MJ et al. 2012. ISRN Pharm. 2012, 718645. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/23304561

"Variations in quantity and quality of essential oil (EO) from the aerial parts of cultivated Mentha piperita were determined……The EO exhibited strong antifungal activities against the examined fungi at concentrations ranging from 0.12 to 8.0 µL/mL. In addition, the EO inhibited the biofilm formation of Candida albicans and C. dubliniensis at concentrations up to 2 µL/mL. Considering the wide range of the antifungal activities of the examined EO, it might be potentially used in the management of fungal infections or in the extension of the shelf life of food products." As taken from Warikoo R et al. 2011. Parasitol. Res. 109(4), 1125-31. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/21445613?dopt=AbstractPlus

"The housefly Musca domestica L. is recognized as a public health pest causing a serious threat to human and livestock by vectoring many infectious diseases.....the essential oils of Mentha

piperita, Zingiber officinalis, Emblica officinalis, and Cinnamomum verum were evaluated for their larvicidal, attractant/repellent, and oviposition attractant/deterrent activity against M. domestica. The highest larvicidal activity, i.e., C(50) = 104 ppm was shown by M. piperita. This oil also exhibited 96.8% repellency at the concentration of 1%. The highest oviposition deterrence activity of 98.1% was also exhibited by M. piperita oil at the concentration of 1%. Among the remaining plants, the essential oil of Z. officinalis exhibited significant bioactivities against M. domestica with larvicidal activity, i.e., lethal concentration (LC)(50) = 137 ppm, repellency of 84.9 and 98.1% oviposition deterrence both at 1% concentration. The other two plant oils, viz., C. verum and E. officinalis, showed relatively moderate bioefficacy with larvicidal activity, i.e., LC(50) = 159 and 259 ppm, repellency of 77.9 and 63.0% while oviposition deterrence of 60.0 and 42.6%, respectively. The result revealed that the essential oils of M. piperita have control potential against M. domestica and should be further explored as a component of integrated vector management program." As taken from Morey RA & Khandagle AJ et al. 2012. Parasitol. Res. 111(4), 1799-805. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22777704

"The toxicity of six plant essential oils to the chewing louse, Bovicola (Werneckiella) ocellatus collected from donkeys, was examined in laboratory bioassays. The oils examined were: tea-tree (Melaleuca alternifolia), lavender (Lavandula angustifolia), peppermint (Mentha piperita), eucalyptus (Eucalyptus globulus Labillardiere), clove bud (Eugenia caryophyllata) and camphor (Cinnamomum camphora). All except camphor oil showed high levels of toxicity, with significant dose-dependent mortality and an LC(50) at concentrations of below 2% (v/v). Hundred percent mortality was achieved at concentrations of 5-10% (v/v). Two essential oil components: eugenol and (+)-terpinen-4-ol showed similar levels of toxicity. The data suggest that these botanical products may offer environmentally and toxicologically safe, alternative veterinary pediculicides for the control of ectoparasitic lice." As taken from Talbert R & Wall R. 2012. Res. Vet. Sci. 93(2), 831-5. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22177577

Record for oils, peppermint:

Spec. Sci. Name Spec. Common Name	**Resp. Site Exp. Dur. (Days)**	**Media Type Test Loc.**	**Exp. Type Chem. Anal.**	**Dose# Res. Sample Unit**	**Endpoint BAF/BCF**	**Effect Effect Meas.**	**Signif. Sig. Level**	**Dose Dose Stat. Meth.**
Trichoderma harzianum Fungus	1	CUL LAB	CM U		LOEL	MOR MORT	SIG <0.05	F (3.0-4.0) ul/ml
Trichoderma harzianum Fungus	MYC 21	CUL LAB	CM U		LOEL	POP ABND	SIG <0.05	F (3.0-4.0) ul/ml
Verticillium fungicola Fungus	MYC 21	FLT LAB	DA U		LOEL	POP ABND	SIG <0.05	F < 5 ul/eu
Verticillium fungicola Fungus	MYC 1	CUL LAB	CM U		LOEL	POP ABND	SIG <0.05	F (2.5-3.5) ul/ml
Penicillium sp. Fungi	3	CUL LAB	CM U		LOEL	POP ABND	SIG <0.05	F 200 ul/ml
Aspergillus niger niger Fungi	3	CUL LAB	CM U		LOEL	POP ABND	SIG <0.05	F 200 ul/ml
Penicillium chrysogenum Fungus	3	CUL LAB	CM U		LOEL	POP ABND	SIG <0.05	F 200 ul/ml

Trametes versicolor Fungus	84	MIX LAB	EN U	2	LOEL	POP ABND	ASIG <0.05	F 300 ul/ml
Penicillium sp. Fungi	3	CUL LAB	CM U		NOEL	POP ABND	NOSIG <0.05	F 100 ul/ml
Aspergillus niger niger Fungi	3	CUL LAB	CM U		NOEL	POP ABND	NOSIG <0.05	F 100 ul/ml
Penicillium chrysogenum Fungus	3	CUL LAB	CM U		NOEL	POP ABND	NOSIG <0.05	F 100 ul/ml
Trametes versicolor Fungus	3	CUL LAB	CM U		NOEL	POP ABND	NOSIG <0.05	F 300 ul/ml
Penicillium sp. Fungi	(28 - 84)	MIX LAB	EN U	2		POP ABND		F 300 ul/ml
Aspergillus niger niger Fungi	(28 - 84)	MIX LAB	EN U	2		POP ABND		F 300 ul/ml
Penicillium chrysogenum Fungus	(28 - 84)	MIX LAB	EN U	2		POP ABND		F 300 ul/ml
Spodoptera frugiperda Fall Armyworm	MI 2	NONE LAB	FD U	2		ENZ AEPX		F .2 %
Coptotermes gestroi Termite	28	MIX LAB	EN U	2		FDB FCNS		F 300 ul/ml
Coptotermes gestroi Termite	28	MIX LAB	EN U	2	NR-LETH	MOR MORT		F 300 ul/ml

As taken from US EPA ECOTOX database

“BACKGROUND: The consequence of misusing chemical biocides in controlling pests and diseases has drawn the attention of policy makers to the development of methods potentially available in nature for this purpose. In the present study the inhibitory effects of black caraway, fennel and peppermint essential oils against Botrytis cinerea were tested at various concentrations in vitro and in vivo. RESULTS: The in vitro results showed that the growth of B. cinerea was completely inhibited by the application of black caraway and fennel oils at concentrations of 400 and 600 μL L(-1) respectively. The in vivo results indicated that black caraway, fennel and peppermint oils at all applied concentrations inhibited B. cinerea growth on plum fruits compared with the control. In addition, all three oils at higher concentrations showed positive effects on fruit quality characteristics such as titrable acidity, total soluble solids, carbohydrate content, pH and weight loss percentage. Thus the oils inhibited the infection of plum fruits by B. cinerea and increased their storage life. CONCLUSION: This research confirms the antifungal effects of black caraway, fennel and peppermint essential oils both in vitro and in vivo on plum fruits postharvest. Therefore these essential oils could be an alternative to chemicals to control postharvest phytopathogenic fungi on plum fruits.” As taken from Aminifard MH and Mohammadi S. 2013. J.

Sci. Food Agric. 93(2), 348-53. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/22740387?dopt=AbstractPlus

“The nematicidal 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 nematicidal 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 nematicidal 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

“The antifungal activity of plant essential oils was evaluated as postharvest treatment on stone fruit against brown rot and grey mold rot of stone fruit caused by Monilinia laxa and Botrytis cinerea, respectively. The essential oils from basil (Ocimum basilicum), fennel (Foeniculum sativum), lavender (Lavandula officinalis), marjoram (Origanum majorana), oregano (Origanum vulgare), peppermint (Mentha piperita), rosemary (Rosmarinus officinalis), sage (Salvia officinalis), savory (Satureja montana), thyme (Thymus vulgaris), and wild mint (Mentha arvensis) were tested at two different concentrations on apricots (cv. Kyoto and cv. Tonda di Costigliole), nectarines (cv. Big Top and cv. Nectaross) and plums (cv. Italia and cv. TC Sun). The volatile composition of the essential oils tested was determined by gas chromatography-mass spectrometry analysis. The treatments containing essential oils from oregano, savory, and thyme at 1% (vol/vol) controlled both B. cinerea and M. laxa growing on apricots cv. Tonda di Costigliole and plums cv. Italia and cv. TC Sun; however, the same treatments were phytotoxic for the carposphere of nectarines cv. Big Top and cv. Nectaross. Treatments with 10% (vol/vol) essential oils were highly phytotoxic, notwithstanding their efficacy against the pathogens tested. The essential oils containing as major components α-pinene, p-cymene, carvacrol, and thymol showed similar results on stone fruit, so their antimicrobial activity and the phytotoxicity produced could be based on the concentration of their principal compounds and their synergistic activity. The efficacy of the essential oil treatments on control of fungal pathogens in postharvest depended on the fruit cultivar, the composition and concentration of the essential oil applied, and the length of storage.” As taken from Lopez-Reyes JG et al. 2013. J. Food Prot. 76(4), 631-9. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23575125

“BACKGROUND: In the last years essential oils from different plants were used in the prevention of fungi and mycotoxins accumulation in cereals. The most attractive aspect derived from using of essential oils as seed grains protectants is due to their non-toxicity. This study was focused on assessment the inhibitory effect of some essential oils: Melissa officinalis (O1), Salvia officinalis (O2), Coriandrum sativum (O3), Thymus vulgaris (O4) Mentha piperita (O5) and Cinnamomum zeylanicum (O6) against natural mycoflora and Fusarium mycotoxins production correlated with their antioxidants properties. RESULTS: All essential oils showed inhibitory effect on fungal contamination of wheat seeds. This ability was dose-dependent. The highest inhibitory effect on Fusarium and Aspergillus fungi was recorded after 5 days of treatment. Fungi such as yeast (Pichia, Saccharomyces and Hyphopichia) were predominantly on seeds mycoflora after 22 days. Each treatment had a selective inhibitory effect on frequency of fungus genera. After 5 days of treatment the most fungicidal effect was recorder for O4, followed by O1. In terms of essential oils effect on mycotoxins development, the best control on fumonisins (FUMO) production was recorded for O6. The antioxidant properties of essential oils decreased in order: O4 > O1 > O6 > O5 > O2 > O3. Also, our data suggested that there is a significant negative correlation between antioxidant properties

and seed contamination index (SCI), but there was not recorded a good correlation between antioxidant properties and FUMO content. CONCLUSIONS: Based on proven antifungal and antimycotoxin effects as well as their antioxidant properties, the essential oils could be recommended as natural preservatives for stored cereals. The highest inhibition of fungal growth was noted after 5 days of treatment and decreased after 22 days. As taken from Sumalan RM et al. 2013. Chem. Cent. J. 7(1):32. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23409841

"BACKGROUND: The study objectives were: (1) to field test potential repellency of common essential oils against several pestiferous social wasps (Hymenoptera: Vespidae), using attractant-baited traps; (2) to identify vespid antennally active compounds from the repellent essential oils; (3) to determine potential repellency of these electroantennographic detection (EAD) active compounds in the field. RESULTS: Of the 21 essential oils tested, 17 showed significant repellency on yellowjackets [mainly Vespula pensylvanica (Saussure)] and paper wasps [mainly Polistes dominulus (Christ)]: clove, pennyroyal, lemongrass, ylang ylang, spearmint, wintergreen, sage, rosemary, lavender, geranium, patchouli, citronella, Roman chamomile, thyme, fennel seed, anise and peppermint. Two essential oil mixtures - 3EO-mix (clove, geranium and lemongrass) and 4EO-mix (clove, geranium, lemongrass and rosemary) - totally blocked the attraction of vespid workers. Twenty-nine vespid antennally active compounds were identified from solid-phase microextraction (SPME) samples of 11 strongly repellent essential oils by GC-EAD/MS techniques. Among the synthetic EAD-active compounds field tested, eugenol, P/I-menthone, pulegone, α/β-thujone, l-carvone, E/Z-citral, citronellal, methyl benzoate, benzyl acetate, methyl salicylate and 3-octanol showed a significant repellency on vespid workers. These compounds are likely responsible for the repellency of their corresponding essential oils. CONCLUSION: These repellent essential oils and their active compositions have great potential for efficient, environmentally sound semiochemical-based IPM of pestiferous vespid wasps." As taken from Zhang QH et al. 2013. Pest. Manag. Sci. 69(4), 542-52. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23081867

"Nasopharyngeal myiasis of camels is caused by the larvae of Cephalopina titillator. We determined the efficacy of essential oils (EOs) of pumpkin, Cucurbita maxima; lupinus, Lupinus luteus; garlic oil, Allium sativum; and peppermint, Mentha piperita, against the third larval stage of C. titillator using larval immersion tests. The positive control group was treated with ivermectin and the negative control one was treated with distilled water and few drops of Tween 80. Larvae were reared until adult emergence. The data indicated that complete larval mortalities were reached 24 h post treatment (PT) with 2 % pumpkin, 7.5 % garlic and peppermint, 30 % lupinus, and 0.15 % ivermectin. The lethal values, LC50s, were 0.20, 0.44, 0.42, 0.47, and 0.03 %, respectively. Pumpkin and ivermectin were 2 and 17 times, respectively, more effective than the other EOs. Ivermectin was seven times more intoxicating than pumpkin oil. Formation of pupae had been stopped after treatment of larvae with 2 % pumpkin, 7.5 % garlic and peppermint, 30 % lupines, and 0.04 % ivermectin. Adult emergence had been completely ceased following treatment of larvae with 0.5 % EOs and 0.04 % ivermectin. Morphological abnormalities were pronounced after treatments, and peppermint oil was the foremost cause of deformation in larvae (44 % PT with 7.5 %) and pupae (40 % PT with 2 %). Pumpkin oil (6 %) was selected to be the drug of choice for controlling C. titillator. Besides their insecticidal effects, EOs are much safer than ivermectin regarding health and environmental issues. Consequently, EOs described herein merit further study as potential nasal drench for C. titillator control." As taken from Khater HF. 2014. Parasitol. Res. 113(2), 593-605. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24276644

ECOSAR version 1.11 reports the following aquatic toxicity data for CAS RN 8006-90-4:

Values used to Generate ECOSAR Profile
Log Kow: 3.381 (EPISuite Kowwin v1.68 Estimate)
Wat Sol: 490 (mg/L, PhysProp DB exp value)

ECOSAR v1.11 Class-specific Estimations
Neutral Organics

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Neutral Organics :	Earthworm	14-day	LC50	195.365

“Greenhouse producers are interested in integrating natural enemies along with pesticides to suppress western flower thrips, Frankliniella occidentalis (Pergande), populations. The insidious flower bug, Orius insidiosus (Say), is a commercially available natural enemy of western flower thrips. We conducted a series of laboratory experiments to determine the direct and indirect effects of 28 pesticides (insecticides, miticides, and fungicides), 4 pesticide mixtures, and 4 surfactants (36 total treatments plus a water control) on the adult O. insidiosus survival and predation on western flower thrips adults under laboratory conditions. The number of live and dead O. insidiosus adults was recorded after 24, 48, 72, and 96 h. The results of the study indicate that the fungicides (aluminum tris, azoxystrobin, fenhexamid, and kresoxim-methyl), insect growth regulators (azadirachtin, buprofezin, kinoprene, and pyriproxyfen), botanicals (Capsicum oleoresin extract, garlic oil, soybean oil; and rosemary, rosemary oil, peppermint oil, and cottonseed oil), and entomopathogenic fungi (Beauveria bassiana and Metarhizium anisopliae) were minimally directly harmful to adult O. insidiosus, with 80% to 100% adult survival. However, abamectin, spinosad, pyridalyl, chlorfenapyr, tau-fluvalinate, imidacloprid, dinotefuran, acetamiprid, and thiamethoxam directly affected O. insidiosus survival after 96 h (0-60% adult survival). The pesticide mixtures of abamectin + spinosad and chlorfenapyr + dinotefuran reduced adult survival (20% and 0%, respectively, after 48 h). Furthermore, the surfactants were not directly harmful to O. insidiosus adults. All western flower thrips adults were killed by the surviving adult O. insidiosus after 48 h, indicating no indirect effects of the pesticides on predation.” As taken from Herrick NJ and Cloyd RA. 2017. J. Econ. Entomol. 110(3), 931-940. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28444217

“The essential oil was obtained by hydrodistillation and the identification and quantification of components were achieved with the use of GC-MS analysis. The antioxidant activity was evaluated by the method of sequestration of DPPH. Essential oils were used for study the cytotoxic front larvae of Artemia salina. In the evaluation of the antimicrobial activity of essential oils, we employed the disk-diffusion method. The potential larvicide in mosquito larvae of the third stage of development of Aedes aegypti to different concentrations of essential oils was evaluated. The major compounds found in the essential oils of M. piperita were linalool (51.8%) and epoxyocimene (19.3%). The percentage of antioxidant activity was 79.9 ± 1.6%. The essential oil showed LC50 = 414.6 µg/mL front of A. saline and is considered highly toxic. It shows sensitivity and halos significant inhibition against E. coli. The essential possessed partial larvicidal efficiency against A. aegypti.” As taken from da Silva Ramos R et al. 2017. Scientific World Journal 2017, 4927214. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28116346

“The larvicidal activity of Mentha piperita, Cymbopogan citratus (lemongrass), Eucalyptus globulus and Citrus sinensis (orange) essential oils and their combinations was evaluated against Musca domestica (housefly) and Anopheles stephensi (mosquitoes) through contact toxicity assay. Among all the tested essential oils/combinations, Me. piperita was found to be the most effective larvicidal agent against Mu. domestica and An. stephensi with LC50 values of 0.66 µl/cm(2) and 44.66 ppm, respectively, after 48 h. The results clearly highlighted that the addition of mentha oil to other oils (1:1 ratio) improved their larvicidal activity. The order of effectiveness of essential oils/combinations indicated that the pattern for An. stephensi follows the trend as mentha > mentha + lemongrass > lemongrass > mentha + eucalyptus > eucalyptus > mentha + orange > orange and for Mu. domestica as mentha > mentha + lemongrass > lemongrass > mentha + orange > orange > mentha + eucalyptus > eucalyptus. The images obtained from scanning electron microscopy (SEM) analysis indicated the toxic effect of Me. piperita as the treated larvae were observed to be dehydrated and deformed. This study demonstrates the effectiveness of tested essential oils/combinations against the larval stages of Mu. domestica and An. stephensi and has the potential for development of botanical formulations.” As taken from Chauhan N et al. 2016. Parasitol. Res. 115(6), 2223-31. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26920567

“Herbal extracts have been investigated as an alternative for parasite control, aiming to slow the development of resistance and to obtain low-cost biodegradable parasiticides. The goal of this study was to evaluate the efficacy, in vitro, of 11 essential oils from Brazil on reproductive efficiency and lethality of the cattle tick Rhipicephalus (Boophilus) microplus. The effects of oils extracted from Curcuma longa, Zingiber officinale, Lippia alba, Lippia gracilis, Lippia origanoides, Lippia sidoides, Mentha arvensis, Mentha piperita, Croton cajucara (white and red), and Croton sacaquinha on ticks were investigated by the Immersion Test with Engorged Females (ITEF) and the modified Larval Packet Test (LPT). Distilled water and 2% Tween 80 were used as control treatments. Chemical analysis of the oils was done with gas chromatography coupled with mass spectrometry. Analysis of the in vitro tests using Probit (SAS program) allowed the calculation of lethal concentrations (LCs). Lower reproductive efficiency indexes and higher efficacy percentages in the ITEF were obtained with the oils extracted from C. longa (24 and 71%, respectively) and M. arvensis oils (27 and 73%, respectively). Lower LC50 was reached with C. longa (10.24 mg/mL), L. alba (10.78 mg/mL), M. arvensis (22.31 mg/mL), L. sidoides (27.67 mg/mL), and C. sacaquinha (29.88 mg/mL) oils. In the LPT, species from Zingiberaceae and Verbenaceae families caused 100% lethality at 25 mg/mL, except for L. sidoides. The most effective oils were from C. longa, L. gracilis, L. origanoides, L. alba, and Z. officinale. The LC50 and LC90 were, respectively: 0.54 and 1.80 mg/mL, 3.21 and 7.03 mg/mL, 3.10 and 8.44 mg/mL, 5.85 and 11.14 mg/mL, and 7.75 and 13.62 mg/mL. The efficacy was directly related to the major components in each essential oil, and the oils derived from Croton genus presented the worst performance, suggesting the absence of synergistic effect among its compounds. Since C. longa, containing 62% turmerone, was the one most efficient against ticks, this compound may be potentially used for tick control, but further research is needed, especially to assess toxicity of these compounds to the host. These new studies, together with the results presented here, may provide a strong rationale for designing pre-clinical and clinical studies with these agents.” As taken from Chagas AC et al. 2016. Ticks Tick Borne Dis. 7(3), 427-32. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26867819

10.5. All other relevant types of ecotoxicity

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that oils, peppermint (CAS RN 8006-90-4) are not bioaccumulative in the environment.

Data accessed July 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:	1.772 (BCF = 59.12 L/kg wet-wt)
Log Biotransformation Half-life (HL):	-0.1483 days (HL = 0.7108 days
Log BCF Arnot-Gobas method (upper trophic):	1.368 (BCF = 23.36)
Log BAF Arnot-Gobas method (upper trophic):	1.368 (BAF = 23.36)
log Kow used:	3.19 (expkow database)

11. References for conventional products

- Adel M et al. (2016). Hemato - Immunological and biochemical parameters, skin antibacterial activity, and survival in rainbow trout (Oncorhynchus mykiss) following the diet supplemented with Mentha piperita against Yersinia ruckeri. Fish Shellfish Immunol. 55, 267-73. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27245867
- Akhavan Amjadi M et al. (2012). The effect of peppermint oil on symptomatic treatment of pruritus in pregnant women. Iran. J. Pharm. Res. 11(4), 1073-7. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24250539

- Alam MS et al. (2013). Efficacy of Peppermint Oil in Diarrhea Predominant IBS - A Double Blind Randomized Placebo - Controlled Study. Mymensingh Med. J. 22(1), 27-30. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/23416804
- Alarie Y [undated]. Unpublished data (cited in Federal Register, 1976).
- Alexa A et al. (2018). Phytochemical Screening and Biological Activity of Mentha × piperita L. and Lavandula angustifolia Mill. Extracts. Anal. Cell Pathol. (Amst). 2018, 2678924. DOI 10.1155/2018/2678924. PubMed, 2018 available at: https://www.ncbi.nlm.nih.gov/pubmed/29552454
- Aminifard MH and Mohammadi S (2013). Essential oils to control Botrytis cinerea in vitro and in vivo on plum fruits. J. Sci. Food Agric. 93(2), 348-53. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/22740387?dopt=AbstractPlus
- Andersen P H & Jensen N J (1984). Mutagenic investigation of peppermint oil in the Salmonella/mammalian-microsome test. Mutation Research, 138, 17-20 (cited in BIBRA, 1999).
- Anheyer D et al. (2017). Herbal Medicines for Gastrointestinal Disorders in Children and Adolescents: A Systematic Review. Pediatrics 139(6), e20170062. DOI 10.1542/peds.2017-0062. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28562281
- Anon (1985). Tokishikoroji Foramu (Toxicology Forum), 8, 91 (cited in RTECS, 2011).
- Arruda MO et al. (2017). The Hydroalcoholic Extract Obtained from Mentha piperita L. Leaves Attenuates Oxidative Stress and Improves Survival in Lipopolysaccharide-Treated Macrophages. J. Immunol. Res. 2017, 2078794. DOI 10.1155/2017/2078794. PubMed, 2018 available at: https://www.ncbi.nlm.nih.gov/pubmed/29085843
- Asao T et al. (2001), Gastrointest Endosc. 2001 Feb;53(2):172-7. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=11174287&query_hl=7&itool=pubmed_docsum
- Ash M (1995). Handbook of food additives: an international guide to more than 7000compounds by trade name, chemical, function and manufacture. Gower Publishing Ltd.ISBN 0-566-07592-x.
- Baker R and Bishop L. (2005). The pyrolysis of non-volatile tobacco ingredients using a system that stimulates cigarette combustion conditions. J. Anal. Appl. Pyrolysis 74, 145–170.
- Baker R et al. (2004a). The effect of tobacco ingredients on smoke chemistry.Part I: Flavourings and additives. Food and Chemical Toxicology 42s, S3-S37.
- Baker R et al. (2004c). An overview of the effects of tobacco ingredients on smoke chemistry and toxicity. Food and Chemical Toxicology 42s, S53-S83.
- Bayat R and Borici-Mazi R (2014). A case of anaphylaxis to peppermint. Allergy Asthma Clin. Immunol. 10(1), 6. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24472564
- Beesley A et al. (1996), Gut. 1996 Aug;39(2):214-9. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=8991859&query_hl=19&itool=pubmed_DocSum
- Begas E et al. (2017). Effects of peppermint tea consumption on the activities of CYP1A2, CYP2A6, Xanthine Oxidase, N-acetyltranferase-2 and UDP-glucuronosyltransferases-1A1/1A6 in healthy volunteers. Food Chem. Toxicol. 100, 80-89. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28011360
- Behrends M et al. (2005), Anesth Analg. 2005 Oct;101(4):1160-2. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=16192538&query_hl=9&itool=pubmed_docsum
- BIBRA (1999). Toxicity Profile: Peppermint oil, powered by Toxnet. URL: http://toxnet.nlm.nih.gov/
- Burdock GA (ed.) (2010). Fenaroli's Handbook of Flavor Ingredients. 6th ed. Boca Raton, FL, p. 1627.
- Caboni P et al. (2013). Nematicidal activity of mint aqueous extracts against the root-knot nematode Meloidogyne incognita. J. Agric. Food Chem. 61(41), 9784-8. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24050256
- Carmines E (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 1.

Cigarette design, testing approach, and review of results. Food and Chemical Toxicology, 40, 77-91.

- CD-ROM 1, JTI Submission, 2002
- Chagas AC et al. (2016). Efficacy of 11 Brazilian essential oils on lethality of the cattle tick Rhipicephalus (Boophilus) microplus. Ticks Tick Borne Dis. 7(3), 427-32. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26867819
- Chauhan N et al. (2016). Larvicidal potential of essential oils against Musca domestica and Anopheles stephensi. Parasitol. Res. 115(6), 2223-31. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26920567
- ChemIDplus. Accessed March 2018. Available at https://chem.nlm.nih.gov/chemidplus/
- Coggins CRE et al. (2011b). A comprehensive evaluation of the toxicology of cigarette ingredients: essential oils and resins. Inhalation Toxicology, 23 (S1), 41-69.
- CosIng (Cosmetic substances and ingredients database). Record for Mentha piperita oil. Undated, accessed March 2018. Available at http://ec.europa.eu/growth/tools-databases/cosing/
- Cosmetics Bench Reference (1996). Published by Cosmetics and Toiletries. ISBN 0-931710-51-0.
- da Silva Ramos R et al. (2017). Chemical Composition and In Vitro Antioxidant, Cytotoxic, Antimicrobial, and Larvicidal Activities of the Essential Oil of Mentha piperita L. (Lamiaceae). Scientific World Journal 2017, 4927214. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28116346
- Dagli N et al. (2015). Essential oils, their therapeutic properties, and implication in dentistry: A review. J. Int. Soc. Prev. Community Dent. 5(5), 335-40. PubMed, 2016 available at: http://www.ncbi.nlm.nih.gov/pubmed/26539382
- Davies SJ et al. (2002), Clin J Pain. 2002 May-Jun;18(3):200-2. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=12048423&query_hl=19&itool=pubmed_DocSum
- de Aguiar FC et al. (2018). Antimicrobial activity of selected essential oils against Streptococcus suis isolated from pigs. Microbiologyopen. Epub ahead of print. DOI 10.1002/mbo3.613. PubMed, 2018 available at: https://www.ncbi.nlm.nih.gov/pubmed/29575822
- DeKoven JG et al. (2017). North American Contact Dermatitis Group Patch Test Results 2013-2014. Dermatitis 28(1), 33-46. DOI 10.1097/DER.0000000000000225. PubMed, 2018 available at: https://www.ncbi.nlm.nih.gov/pubmed/27775967
- Department of Health (2003). Permitted Additives to Tobacco Products in the United Kingdom. Department of Health, London. October 2003. Available at: http://webarchive.nationalarchives.gov.uk/20130107105354/http://www.dh.gov.uk/prod_consum_dh/groups/dh_digitalassets/@dh/@ab/documents/digitalasset/dh_095251.pdf
- Dew MJ et al. (1984), Br J Clin Pract. 1984 Nov-Dec;38(11-12):394, 398. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=6397219&query_hl=1&itool=pubmed_DocSum
- Doull et al. (1994). A safety assessment of the ingredients added to tobacco in the manufacture of cigarettes. Available at http://legacy.library.ucsf.edu/tid/thy03c00
- Doull et al. (1998). A safety assessment of the ingredients added to tobacco in the manufacture of cigarettes. Available at http://legacy.library.ucsf.edu/tid/wzp67e00
- Douros A et al. (2016). Herb-Induced Liver Injury in the Berlin Case-Control Surveillance Study. Int. J. Mol. Sci. 17(1), E114. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26784183
- Dresser GK et al. (2002), Clin Pharmacol Ther. 2002 Sep;72(3):247-55. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=12235445&query_hl=5&itool=pubmed_docsum
- EAFUS (2013). US Food and Drug Administration. Everything added to food in the United States. Last updated 23 April 2013. Accessed March 2018. Available at https://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=eafusListing
- ECHA (2016). European Chemicals Agency. Annex III Inventory. Last updated 18 May 2016.

Available at: https://echa.europa.eu/information-on-chemicals/annex-iii-inventory

- ECHA (2018a). European Chemicals Agency. Information on Chemicals. Record for oils, peppermint (CAS RN 8006-0-4). Last updated 7 February 2018. Available at: https://echa.europa.eu/information-on-chemicals/pre-registered-substances
- ECHA (2018b). European Chemicals Agency. Information on Chemicals. Record for peppermint, ext. (CAS RNs 8006-90-4/84082-70-2). Last updated 27 March 2018. Available at: https://echa.europa.eu/information-on-chemicals/registered-substances
- ECHA (2018c). European Chemicals Agency. Classification and Labelling (C&L) Inventory database. Last updated 28 March 2018. Available at: http://echa.europa.eu/information-on-chemicals/cl-inventory-database
- ECOSAR. Record for oils, peppermint (CAS RN 8006-90-4). Accessed July 2017. (ECOSAR content has not been updated since 2012, version 1.11.) Available to download, through EPISuite, at https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface
- EMA (2014). European Medicines Agency. Public statement on the use of herbal medicinal products containing pulegone and menthofuran. Draft revision 1. 24 November 2014. EMA/HMPC/138386/2005 Rev. 1. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Public_statement/2014/12/WC500179556.pdf
- EMEA (2008). European Medicines Agency. Assessment report on *Mentha x piperita* l., Aetheroleum. Available at http://www.emea.europa.eu/docs/en_GB/document_library/Herbal_-_HMPC_assessment_report/2010/01/WC500059311.pdf
- EPISuite (undated). Record for oils, peppermint (CAS RN 8006-40-9). Accessed July 2017. (EPISuite content has not been updated since 2012, version 4.11.) The programme is available to download via https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface
- EPISuite (2017). Record for oils, peppermint (CAS RN 8006-90-4). EPISuite version 4.11. Last updated June 2017. EPISuite is available to download at https://www.epa.gov/tsca-screening-tools/download-epi-suitetm-estimation-program-interface-v411
- Fashner J and Gitu AC (2013). Common gastrointestinal symptoms: irritable bowel syndrome. Family Physician Essentials 413, 16-23. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24124703
- FDA (2018). US Food and Drug Administration. Electronic Code of Federal Regulations (e-CFR), Title 21. Current as of 26 March 2018. Accessed March 2018. Available at: https://www.ecfr.gov/cgi-bin/ECFR?page=browse
- Ferreira P et al. (2014). Mentha piperita essential oil induces apoptosis in yeast associated with both cytosolic and mitochondrial ROS-mediated damage. FEMS Yeast Res. 14(7), 1006-1014. PubMed, available at http://www.ncbi.nlm.nih.gov/pubmed/25065265
- fGLH Study Report (2010).
- Fox N (1930). Archs Otolar. 11, 48.
- Gaworski C.L. et al. (1998). Toxicologic evaluation of flavor ingredients added to cigarette tobacco: 13-week inhalation exposures in rats. Inhalation Toxicology, 10:357-381.
- Gaworski C.L. et al. (1999). Toxicologic evaluation of flavor ingredients added to cigarette tobacco: skin painting bioassay of cigarette smoke condensate in SENCAR mice. Toxicology 139, 1-17.
- Gaworski CL et al. (2011a). An evaluation of the toxicity of 95 ingredients added individually to experimental cigarettes: approach and methods. Inhalation Toxicology, 23 (S1), 1-12.
- Gaworski CL et al. (2011b). Insights from a multi-year program designed to test the impact of ingredients on mainstream cigarette smoke toxicity. Inhalation Toxicology, 23 (S1), 172-183.
- Grigoleit HG & Grigoleit P. (2005) Phytomedicine. Aug;12(8):612-6. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=16121523&query_hl=19&itool=pubmed_DocSum
- Grigoleit HG & Grigoleit P. (2005) Phytomedicine. Aug;12(8):607-11. PubMed, 2010 available at

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=16121522&query_hl=19&itool=pubmed_DocSum

- Hall RL and Oser BL (1965). Recent progress in the consideration of flavoring ingredients under the Food Additives Amendment. III. GRAS substances. Food Technology, 19, 151-197. Available at https://www.femaflavor.org/sites/default/files/3.%20GRAS%20Substances%282001-3124%29_0.pdf
- Hamoud R et al. (2012). Antimicrobial activity of a traditionally used complex essential oil distillate (Olbas(®) Tropfen) in comparison to its individual essential oil ingredients. Phytomedicine 19(11), 969-76. Pubmed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22739414
- Haresaku M et al (1985). Mutagenicity study (Ames test) of toothpaste ingredients.Journal of the Society of Cosmetic Chemists, Japan, 19, 100-104 (in Japanese).
- Hassan HA et al. (2013). Mentha piperita as a pivotal neuro-protective agent against gamma irradiation induced DNA fragmentation and apoptosis: Mentha extract as a neuroprotective against gamma irradiation. Cytotechnology 65(1), 145-56. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/23011739
- Heck DE et al. (1989). An evaluation of food flavoring ingredients in a genetic toxicity screening battery. The Toxicologist, 9(1), 257.
- Herrick NJ and Cloyd RA (2017). Direct and Indirect Effects of Pesticides on the Insidious Flower Bug (Hemiptera: Anthocoridae) Under Laboratory Conditions. J. Econ. Entomol. 110(3), 931-940. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28444217
- Hiki N et al. (2003), Gastrointest Endosc. 2003 Apr;57(4):475-82. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=12665756&query_hl=19&itool=pubmed_DocSum
- Hilliard CA, et al. (1998) Chromosome aberrations in vitro related to cytotoxicity of nonmutagenic chemicals and metabolic poisons.Environ Mol Mutagen. 1998;31(4):316-26.
- HSDB (2003). Peppermint oil. Last updated 14 February 2003. Accessed March 2018. Available at http://toxnet.nlm.nih.gov/newtoxnet/hsdb.htm
- IFRA (2016). International Fragrance Association. IFRA Volume of Use Survey 2016: Transparency List. Accessed March 2018. Available at http://www.ifraorg.org/en-us/ingredients#.Ws8KmTtwYfl
- Ishidate M et al. (1984). Primary mutagenicity screening of food additives currently used in Japan. Food and Chemical Toxicology, 22, 623-636.
- Ishidate M et al. (1988). A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures. Mutation Research, 195, 151-213 (cited in BIBRA, 1999).
- Jack AR et al. (2013). Allergic contact dermatitis to plant extracts in cosmetics. Semin. Cutan. Med. Surg. 32(3), 140-6. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24175401
- JECFA (1999). Safety evaluation of certain food additives. WHO Fd Add. Ser. 42. Prepared by the Fifty-First Meeting of the Joint FAO/WHO Expert Committee on Food Additives.
- JTI KB Study Reports (s).
- JTI Study Report (s).
- Kalavala M et al. (2007). Allergic contact dermatitis to peppermint foot spray. Contact Dermatitis, 57, 57-58.
- Kearns GL et al. (2015). Systemic exposure to menthol following administration of peppermint oil to paediatric patients. BMJ Open 5(8), e008375. PubMed, 2016 available at: http://www.ncbi.nlm.nih.gov/pubmed/26270949
- Khan IA and Abourashed EA (2010). Leung's Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics. Third Edition. John Wiley & Sons, Inc., Hoboken, New Jersey.
- Khanna R et al. (2014). Peppermint Oil for the Treatment of Irritable Bowel Syndrome: A Systematic Review and Meta-analysis. J. Clin. Gastroenterol. 48(6), 505-12. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24100754

- Khater HF (2014). Bioactivities of some essential oils against the camel nasal botfly, Cephalopina titillator. Parasitol. Res. 113(2), 593-605. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24276644
- Koo HN et al. (2001), J Mol Neurosci. 2001 Dec;17(3):391-6. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=11859935&query_hl=19&itool=pubmed_DocSum
- Korterink JJ et al. (2015). Pharmacologic treatment in pediatric functional abdominal pain disorders: a systematic review. J. Pediatr. 166(2), 424-31. PubMed, 2016 available at: http://www.ncbi.nlm.nih.gov/pubmed/25449223
- Koutroumanidou E et al. (2013). Increased seizure latency and decreased severity of pentylenetetrazol-induced seizures in mice after essential oil administration. Epilepsy Res. Treat. 2013, 532657. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23819045
- Kowalski Z et al. (1962). Medycyna Pracy, 13, 69.
- Kuroda K et al. (1989). Seikatsu Eisei, 33, 15 (cited in BIBRA, 1999).
- Lazutka J R et al. (2001). Genotoxicity of dill (Anethum graveolens L.), peppermint (Mentha x piperita L.) and pine (Pinus sylvestris L.) essential oils in human lymphocytesand Drosophila melanogaster. Food and Chemical Toxicology, 39, 485-492.
- LBI (1973). Summary of mutagenicity screening studies. FDA Compound 71-57. Menthol. Contract FDA-71-628. Litton Bionetics Inc., Bethesda, Maryland.
- LBI (1975). Mutagenic evaluation of FDA Compound 71-57. Menthol. Report PB-245444. Litton Bionetics Inc., Bethesda, Maryland (cited in JECFA, 1999).
- Leung A. & Foster S., Encyclopaedia of common natural ingredients used in food, drugs and cosmetics, 2nd edition, 2003, pp. 368-372
- Li J et al. (2011). Peppermint oil decreases the production of virulence-associated exoproteins by Staphylococcus aureus. Molecules 16(2), 1642-54. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/21326141?dopt=AbstractPlus
- Liakos I et al. (2013). All-natural composite wound dressing films of essential oils encapsulated in sodium alginate with antimicrobial properties. Int. J. Pharm. 463(2), 137-45. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24211443
- Lloyd R A et al. (1976). Flue-cured tobacco flavour. 1. Essence and essential oil components. Tobacco Science, 20, 40-48.
- Lopez-Reyes JG et al. (2013). Efficacy of plant essential oils on postharvest control of rots caused by fungi on different stone fruits in vivo. J. Food Prot. 76(4), 631-9. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23575125
- Luke E. (1962). Lancet i, 110.
- Mahmoud I et al. (1992). Mutagenic and toxic activities of several spices and some Jordanian medicinal plants. International Journal of Pharmacognosy, 30, 81-85.
- Marjani A et al. (2012). Effect of peppermint oil on serum lipid peroxidation and hepatic enzymes after immobility stress in mice. Open Biochem. J. 6, 51-5. PubMed, 2013, available at http://www.ncbi.nlm.nih.gov/pubmed/22654997
- Martindale (1993). The Extra Pharmacopoeia. Edited by J E F Reynolds. Thirteenth edition. The Pharmaceutical Press. ISBN 0-85369-300-5.
- Meamarbashi A and Rajabi A (2013). The effects of peppermint on exercise performance. J. Int. Soc. Sports Nutr. 10(1), 15. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23517650
- Merck (2013). The Merck Index. An encyclopaedia of chemicals, drugs and biologicals. Fifteenth edition. O'Neil MJ et al ed. Merck and Co., Inc.; Whitehouse Station, New Jersey, USA. ISBN 978-1-84973-670-1
- Mogosan C et al. (2017). A Comparative Analysis of the Chemical Composition, Anti-Inflammatory, and Antinociceptive Effects of the Essential Oils from Three Species of Mentha Cultivated in Romania.

Molecules 22(2), E263. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28208614

- Morey RA & Khandagle AJ et al. (2012). Bioefficacy of essential oils of medicinal plants against housefly, Musca domestica L. Parasitol. Res. 111(4), 1799-805. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22777704
- Morton CA et al. (1995), Contact Dermatitis. 1995 May;32(5):281-4. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=7634781&query_hl=13&itool=pubmed_docsum
- Muhammad F et al. (2017). Influence of some plant extracts on the transdermal absorption and penetration of marker penetrants. Cutan. Ocul. Toxicol. 36(1), 60-66. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27027912
- Nath SS et al. (2012). A near fatal case of high dose peppermint oil ingestion- Lessons learnt. Indian J. Anaesth. 256(6), 582-4. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23325948
- NCI (1978). Bioassay of dl-menthol for possible carcinogenicity. Report PB-288 761. National Cancer Institute, Bethesda, Maryland.
- NIOSH. National Institute for Occupational Safety and Health. National Occupational Exposure Survey (1981-1983). Record for oil, peppermint (CAS RN 8006-90-4). Available at https://web.archive.org/web/20111028111422/http://www.cdc.gov/noes/noes2/80680occ.html
- NTP, (2011), NTP Technical Report on the Toxicology and Carcinogenesis Studies of Pulegone (CAS NO. 89-82-7) in F344/N Rats and B6C3F1 Mice (Gavage Studies). NTP TR 563. NIH Publication No. 11-5905. August 2011. National Institutes of Health, Public Health Service, U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES. Available at https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr563.pdf
- NZ EPA (2006). New Zealand Environmental Protection Authority. Inventory of Chemicals. Records for peppermint, ext. (CAS RN 84082-70-2) and oils, peppermint (CAS RN 8006-90-4). Date added to inventory: 1 December 2006. Accessed March 2018. Available at: https://www.epa.govt.nz/database-search/new-zealand-inventory-of-chemicals-nzioc/view/7556 and https://www.epa.govt.nz/database-search/new-zealand-inventory-of-chemicals-nzioc/view/7713
- NZ EPA CCID (undated). New Zealand Environmental Protection Authority. Chemical Classification and Information Database. Record for oils, peppermint (CAS RN 8006-90-4). Accessed March 2018. Available at https://www.epa.govt.nz/database-search/chemical-classification-and-information-database-ccid/view/7713
- OECD. Organization for Economic Co-operation and Development. The Global Portal to Information on Chemical Substances (eChemPortal). Oils, peppermint (CAS RN 8006-90-4). Accessed July 2017. Available via http://www.echemportal.org/echemportal/page.action?pageID=9
- Olsen P., Thorup I. (1984). Neurotoxicity in rats dosed with peppermint oil and pulegone. Disease, Metabolism and reproduction in the toxic response to drugs and other chemicals Arch. Oxicol., Suppl. 7, 408-409.
- O'Mullane N M et al. (1982). Adverse CNS effects of menthol-containing olbas oil. Lancet i, 1121.
- Papathanasopoulos A et al. (2013). Effect of acute peppermint oil administration on gastric sensorimotor function and nutrient tolerance in health. Neurogastroenterol. Motil. 25(4), e263-71. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/23489975
- Pasha H et al. (2012). Study of the effect of mint oil on nausea and vomiting during pregnancy. Iran. Red Crescent Med. J. 14(11), 727-30. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23396673
- Pellegrini M et al. (2018). Characterization of Essential Oils Obtained from Abruzzo Autochthonous Plants: Antioxidant and Antimicrobial Activities Assessment for Food Application. Foods 7(2), E19. DOI 10.3390/foods7020019. PubMed, 2018 available at: https://www.ncbi.nlm.nih.gov/pubmed/29393893
- Pilipenko VI et al. (2013). Contemporary dietotherapy of the irritable bowel syndrome. Vopr. Pitan. 82(1), 64-73. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23808281?dopt=AbstractPlus
- Posadzki P et al. (2013). Adverse effects of herbal medicines: an overview of systematic reviews.

Clin. Med. 13(1), 7-12. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23472485

- Rakieten N et al. (1954). Journal of the American Pharmaceutical Association, 43, 390.
- Renne R et al. (2006). Effects of Flavoring and Casing Ingredients on the Toxicity of Mainstream Cigarette Smoke in Rats. Inhalation Toxicology, 18:685-706.
- Roe F J C et al. (1979). Journal of Environmental Pathology and Toxicology, 2, 799.
- Roemer E et al. (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 3: In vitro genotoxicity and cytotoxicity. Food and Chemical Toxicology, 40, 105-111.
- Roemer E et al., (2014). Toxicological assessment of kretek cigarettes Part 6: The impact of ingredients added to kretek cigarettes on smoke chemistry and in vitro toxicity. Regulatory Toxicology and Pharmacology 70; S66-80.
- Roth TL et al. (2013). Neurobiology of secure infant attachment and attachment despite adversity: a mouse model. Genes Brain Behav. 12(7), 673-80. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23927771
- Roussos AP and Hirsch AR (2014). Alliaceous migraines. Headache 54(2), 378-82. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23551212
- RTECS (2011). Registry of Toxic Effects of Chemical Substances. Peppermint oil (CAS RN 8006-90-4). RTECS number: #SC6125000. Last updated October 2011. Accessed March 2018.
- Rustemeier K et al. (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 2. Chemical composition of mainstream smoke. Food and Chemical Toxicology, 40, 93-104.
- Saharkhiz MJ et al. (2012). Chemical Composition, Antifungal and Antibiofilm Activities of the Essential Oil of Mentha piperita L. ISRN Pharm. 2012, 718645. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/23304561
- Samojlik I et al. (2012). Acute and chronic pretreatment with essential oil of peppermint (Mentha × piperita L., Lamiaceae) influences drug effects. Phytother. Res. 26 (6), 820-5. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22076909?dopt=AbstractPlus
- Sasaki Y F *et al.* (2000). The comet assay with multiple mouse organs: comparison of comet assay results and carcinogenicity with 208 chemicals selected from the IARC monographs and U.S. NTP Carcinogenicity Database. CRC Critical Reviews in Toxicology, 30, 629-799.
- SCCS (2011). Scientific Committee on Consumer Safety (SCCS). Opinion on fragrance allergens in cosmetic products. Pre-consultation opinion adopted by the SCCS at its 13th plenary meeting of 13-14 December 2011. Available at http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_073.pdf
- Schievelbein H (1969). Munch. Med. Wschr. 111, 2457.
- Schramke H et al., (2014). Toxicological assessment of kretek cigarettes Part 7: The impact of ingredients added to kretek cigarettes on inhalation toxicity. Regulatory Toxicology and Pharmacology 70; S81-89.
- Shelby M D et al. (1993). Evaluation of a three-exposure mouse bone marrow micronucleus protocol: results with 49 chemicals. Environmental and Molecular Mutagenesis, 21, 160-179.
- Shepherd K and Peart DJ (2017). Aerobic capacity is not improved following 10-day supplementation with peppermint essential oil. Appl. Physiol. Nutr. Metab. 42(5), 558-561. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28177705
- Sites DS et al. (2014). Controlled Breathing With or Without Peppermint Aromatherapy for Postoperative Nausea and/or Vomiting Symptom Relief: A Randomized Controlled Trial. J. Perianesth. Nurs. 29(1), 12-9. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24461278
- Sivaswamy S N et al. (1991). Mutagenic activity of South Indian food items. Indian Journal of Experimental Biology, 29, 730-737.
- Solà-Bonada N et al. (2012). 1.6% peppermint oil solution as intestinal spasmolytic in retrograde endoscopic cholangiopancreatography. Farm. Hosp. 36(4), 256-60. PubMed, 2013 available at

http://www.ncbi.nlm.nih.gov/pubmed/22137159?dopt=AbstractPlus

- Stedman, R L (1968). The Chemical composition of Tobacco and Tobacco Smoke. Chemical Reviews, 68 (2), 153-207.
- Sumalan RM et al. (2013). Assessment of inhibitory potential of essential oils on natural mycoflora and Fusarium mycotoxins production in wheat. Chem. Cent. J. 7(1), 32. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23409841
- Taher YA (2012). Antinociceptive activity of Mentha piperita leaf aqueous extract in mice. Libyan J. Med. 2012, 7. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22468149
- Talbert R & Wall R (2012). Toxicity of essential and non-essential oils against the chewing louse, Bovicola (Werneckiella) ocellatus. Res. Vet. Sci. 93(2), 831-5. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22177577
- Tran A et al. (2010). Acute allergic contact dermatitis of the lips from peppermint oil in a lip balm. Dermatitis 21, 111-115. PubMed available at http://www.ncbi.nlm.nih.gov/pubmed/20233551?dopt=AbstractPlus
- Travassos AR et al. (2011). Non-fragrance allergens in specific cosmetic products. Contact Dermatitis 65, 276-285.
- Umezu T. Pharmacol Biochem Behav. 2010, Feb; 94(4):497-502. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=retrieve&db=pubmed&list_uids=19917306&dopt=AbstractPlus
- Umezu T. (2012). Evaluation of the effects of plant-derived essential oils on central nervous system function using discrete shuttle-type conditioned avoidance response in mice. Phytother. Res. 26(6), 884-91. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22086772
- US Department of Health and Human Services (2017). Household Products Database. Last updated September 2017. Accessed March 2018. Available at https://hpd.nlm.nih.gov/index.htm
- US EPA 2012 CDR list (Chemical Data Reporting Rule). Accessed March 2018. Available at https://iaspub.epa.gov/sor_internet/registry/substreg/searchandretrieve/searchbylist/search.do
- US EPA ECOTOX Database. Record for oils, peppermit (CAS RN 8006-90-4). Accessed December 2014. Available at http://cfpub.epa.gov/ecotox/quick_query.htm
- US EPA Inert Finder Database (2018). Last updated 2 January 2018. Accessed March 2018. Available at https://iaspub.epa.gov/apex/pesticides/f?p=INERTFINDER:1:0::NO:1::.
- US EPA TSCA inventory. Accessed March 2018. Available at https://iaspub.epa.gov/sor_internet/registry/substreg/searchandretrieve/searchbylist/search.do
- Uter W et al. (2010). Contact allergy to essential oils: current patch test results (2000-2008) from the Information Network of Departments of Dermatology (IVDK). Contact Dermatitis 63, 277-283.
- Uzair B et al. (2017). Essential oils showing in vitro anti MRSA and synergistic activity with penicillin group of antibiotics. Pak. J. Pharm. Sci. 30(5(Supplementary)), 1997-2002. PubMed, 2018 available at: https://www.ncbi.nlm.nih.gov/pubmed/29105634
- van Tilburg and Felix CT (2013). Diet and functional abdominal pain in children and adolescents. J. Pediatr. Gastroenterol. Nutr. 57(2), 141-8. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23698023
- Vanscheeuwijck P.M. et al. (2002). Evaluation of the potential effects of ingredients added to cigarettes.Part 4: subchronic inhalation toxicity. Food and Chemical Toxicology 40 (2002) 113-131
- Varney E and Buckle J (2013). Effect of inhaled essential oils on mental exhaustion and moderate burnout: a small pilot study. J. Altern. Complement. Med. 19(1), 69-71. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23140115
- Vermatt H et al. (2008). Vulval allergic contact dermatitis due to peppermint oil in herbal tea. Contact Dermatitis 58, 364-365
- Vo LT et al. (2003), Clin Exp Pharmacol Physiol. 2003 Oct;30(10):799-804. PubMed, 2010 available at

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=14516421&query_hl=3&itool=pubmed_docsum

- Vollmuth TA et al. (1990). An evaluation of food flavoring ingredients using an in vivo reproductive and developmental toxicity screening test. Teratology 41(5), 597.
- Warikoo R et al. (2011). Oviposition-altering and ovicidal potentials of five essential oils against female adults of the dengue vector, Aedes aegypti L. Parasitol. Res. 109(4), 1125-31. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/21445613?dopt=AbstractPlus
- WHO Food Additives Series 42, (2006), available at http://www.inchem.org/documents/jecfa/jecmono/v042je21.htm
- WHO Food Additives Series 46, available at http://www.inchem.org/documents/jecfa/jecmono/v46je10.htm
- Wu JF et al. (2010). Bioequivalence evaluation of menthol after oral administration of peppermint oil soft capsules in dogs. Arzneimittelforschung 60, 479-482. PubMed available at http://www.ncbi.nlm.nih.gov/pubmed/20863003?dopt=AbstractPlus
- Yap PS et al. (2013). Combination of essential oils and antibiotics reduce antibiotic resistance in plasmid-conferred multidrug resistant bacteria. Phytomedicine 20(8-9), 710-3. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23537749
- Zhang QH et al. (2013). Essential oils and their compositions as spatial repellents for pestiferous social wasps. Pest. Manag. Sci. 69(4), 542-52. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23081867

12. Other information

- Klus H et al. 2012. Influence of Additives on Cigarette Related Health Risks. Beiträge zur Tabakforschung 25(3), 412–493. Available at: http://www.degruyter.com/view/j/cttr.2012.25.issue-3/cttr-2013-0921/cttr-2013-0921.xml?rskey=qUDq5B&result=1

- Chumpitazi BP et al. (2018). Review article: the physiological effects and safety of peppermint oil and its efficacy in irritable bowel syndrome and other functional disorders. Aliment. Pharmacol. Ther. 47(6), 738-752. DOI 10.1111/apt.14519. PubMed, 2018 available at: https://www.ncbi.nlm.nih.gov/pubmed/29372567

13. Last audited

April 2018

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.).

Scientific Committee members

Contact
European Commission
Health & Consumers
Directorate D: Health Systems and Products
Unit D5 - Risk Assessment
Office: B232 B-1049 Brussels
Sanco-SCCS-Secretariat@ec.europa.eu

ISSN 1831-
Doi:10.2773/
ISBN 978-92-79-
ND-

Acknowledgements

Dr. C. Chambers
Dr. Q. Chaudry
Dr. S.C. Rastogi
Dr. I.R. White (chairman)

External experts

Prof.. A. Börje University of Gothenburg, Sweden
Prof. J. D. Johansen Gentofte Hospital, University of Copenhagen, Denmark
Prof. A-T. Karlberg University of Gothenburg, Sweden
Prof. C. Lidén Karolinska Institutet, Sweden
Dr. D.W. Roberts Liverpool John Moores University, UK
Prof. W. Uter (rapporteur) Friedrich-Alexander University (FAU), Erlangen, Germany

Keywords: SCCS, scientific opinion, labelling, fragrance allergens, directive 76/768/ECC

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), opinion on fragrance allergens in cosmetic products, 26-27 June 2012

Table of contents

Summary

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

1. Background

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

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

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

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

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

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

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

Furthermore, two fragrances (natural mixtures) were added

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

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

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

2. Terms of reference

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

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

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

3. Introduction

Fragrance ingredients

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

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

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

4. Clinical aspects of contact allergy to fragrance ingredients

4.1. Spectrum of reactions

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

4.1.1. Allergic contact dermatitis

Mechanism

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

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

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

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

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

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

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

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

Overview of clinical features

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

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

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

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

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

Hands

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

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

Axillae

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

Face

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

4.1.2. Irritant reactions (including contact urticaria)

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

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

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

4.1.3. Pigmentary anomalies

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

4.1.4. Photo-reactions

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

4.1.5. General/respiratory

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

4.2. Patch testing

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

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

4.3. Epidemiology of fragrance allergy

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

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

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

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

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

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

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

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

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

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

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

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

4.3.2. Clinical epidemiology

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

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

Notwithstanding the potential pitfalls of clinical data, they have proven useful in identifying emerging trends or persisting problems, and also in evaluating the effect of preventive action – either regarding the entire population, or subgroups thereof, such as certain occupations. Regarding the fragrance mixes (FM I and FM II) mentioned above, evidence regarding sensitisation frequencies published since 1999 will be outlined below, thus supplementing the data presented in the SCCNFP opinion on Fragrance Allergy in 1999 (1).

Fragrance Mix I ("Larsen Mix")

Table 4-3: Results with screening agents for contact allergy to fragrance ingredients reported since 1999 in patients patch tested for suspected allergic contact dermatitis in Europe: Fragrance Mix "I" (see Table 4-1). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS (§).

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI)
Sweden (38)	Consecutive patients	2000	3790	6.9
Hungary (39)		1998-1999	3604	8.2 (7.3–9.1)§
Czech Republic (40)		1997-2001	12058	5.8 (5.4–6.2)§
Ljubljana, Slovenia (41)	Consecutive patients	1989-1998	6129	5.9 (5.3–6.5)§
Germany (42)	Consecutive IVDK patients	1996-2002	59298	11.3 (11.0–11.5)§
Germany (43)	Consecutive IVDK patients	2005-2008	36961	7.3 (7.0–7.6)§
Vienna, Austria (16)	Consecutive patients of one clinic	1997-2000	2660	9.1 (8.1–10.3)§
Groningen, Netherlands (44)	Patients (fragrance allergy suspected)	04/2005-06/2007	295	5.8 (3.4–9.1)§
The Netherlands (45)	Consecutive patients	09/1998-04/1999	1825	10.6 (9.2–12.1)
The Netherlands (46)	Patients (cosmetic allergy suspected)	1994-1998	757	14.8 (12.3–17.5)§
Leuven, Belgium (47)	Consecutive patients	1990-2005	10128	9.1 (8.6–9.7)§
Coimbra, Portugal (48)	Consecutive patients	07/1989-06/1999	2600	10.9 (9.7–12.2)§
Spain (49)	Consecutive patients	10/2005-06/2008	1253	4.5 (3.4–5.8)§
Sheffield, UK (50)	Consecutive patients	1994-1995	744	11.4 (9.2–13.9)§
St. John's, London, UK (51)	Consecutive patients	1980-2004	34072	7.7 (7.4–8.0)§
Copenhagen, Denmark (52)	Consecutive patients	1985-2007	16173	7.2 (6.8–7.6)§
ESSCA (53)	Consecutive patients	2002-2003	9663	7.1 (6.6–7.6)§
ESSCA (54)	Consecutive patients	2004	9941	7.6 (7.1–8.2)§
ESSCA (55)	Consecutive patients	2005-2006	18542	7.0 (6.6–7.4)§

Table 4-4: Results with screening agents for contact allergy to fragrance ingredients reported since 1999 in patients patch tested for suspected allergic contact dermatitis in non-European countries: Fragrance Mix "I" (see Table 4-1). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS ([§]).

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI)
South Korea (56)	Consecutive patients	04/2002–06/2003	422	9.7 (7.1–13.0)[§]
Lahore, Pakistan (57)	Dermatitis patients	2 years prior to 2002	350	7.7 (5.2–11.0)[§]
Manipal, India (58)	Dermatitis patients	1989-1998	1780	3.1 (2.3–4.0)[§]
Tel Aviv, Israel[$](59)	Consecutive patients	1999-2000	943	8.5 (6.8–10.5)[§]
Tel Aviv, Israel (60)	Consecutive patients	1998-2004	2156	7.1 (6.1–8.3)[§]
Tehran, Iran (61)	Consecutive patients	2002-2004	250	4.0 (1.9–7.2)[§]
Ankara, Turkey (62)	Consecutive patients	1992-2004	1038	2.1 (1.3–3.2) [§]
Beijing, China (63)	Consecutive patients	2000-2003	378	15.9 (12.3–20.0)[§]
USA (Canada) (64)	Probably consecutive patients	2003	1603	5.9
NACDG 2009 (US and Canada) (65)	Consecutive patients	2005-2006	4439	11.5

Note: $ Possibly included in (60).

Beyond the studies discussed above, regarding a time trend of sensitisation to FM I, a significant increase of positive results to FM I until 1998, and a significant drop thereafter has been noted in the IVDK study covering 1996 to 2002 (42). A similar drop from 1999 to 2007 has been observed in female, but not male patients from Copenhagen (52). In accordance with these findings, the prevalence of positive reactions to FM I doubled, or thereabouts, from 1989-1993 to 1994-1998 in Ljubljana, Slovenia (41).

Within Europe, a comparison between different countries and clinical departments is possible. An EECDRG study covering 1996-2000 found 9.7% positives to FM I (range: 5.0–12.6% in ten departments from seven European countries (66). A different European study, covering 10/1997-10/1998, found 11.3% (95% CI: 9.9–12.9%) positive reactions to FM 1 in 1,855 patients; the variation between centres was marked: Gentofte 8.2% vs. Leuven 23.0% as extremes (67). In the first study of the European Surveillance System on Contact Allergies (ESSCA), covering 2002 and 2003, 9663 patients were patch tested with FM I, overall yielding 7.1% positive reactions with marked variation between participating departments. In Dortmund, Germany, the minimum frequency of 3.7% was noted, while in Lahti, Finland, the highest prevalence, namely 10.4%, was found (53). Subsequently, in the year 2004, the overall prevalence was 7.6%, i.e. largely unchanged (54). In the most recent study by ESSCA, based on 2005/2006 PT data across Europe, significant differences were again noted, this time on the aggregated level of European regions, with FM I sensitisation being the least frequent in the Southern countries (4.8% [95% CI: 3.9–5.5%] age- and sex-standardised prevalence) vs. 7.7% (95% CI: 7.0–8.4%) in the central European departments, with the Finnish, Polish and Lithuanian departments (5.7% [95%

CI: 4.6 – 6.8%]) and the UK network (6.8% [95% CI: 6.3 – 7.3%]) in an intermediate position (55).

Fragrance Mix II

Table 4-5: Results with screening agents for contact allergy to fragrance ingredients reported since 1999 in patients patch tested for suspected allergic contact dermatitis: Fragrance Mix "II" (see Table 4-2). The FM II was only conceived in 2005, so results are still sparse). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS (§).

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI)
EU (35)	Six clinical depts.	10/2002-06/2003	1701	2.9 (2.2–3.9)§
IVDK, Germany (68)	Consecutive patients	01/2005-12/2008	35633	4.9 (4.7–5.1)§
Groningen, Netherlands (44)	Patients (fragrance allergy suspected)	04/2005-06/2007	227	9.3 (5.8–13.8)§
Leuven, Belgium (47)	Consecutive patients	2005 only	335	2.1 (0.8–4.3)§
Spain (49)	Consecutive patients	10/2005-06/2008	1253	0.6 (0.2–1.1)§
Denmark (69) on behalf of the DCDG, 2010	Consecutive patients	2005-2008	12302	4.5 (4.1–4.9)§

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) has been the most frequently reported chemical causing fragrance allergy since the 1999 opinion on fragrance allergy. In total, reports of about 1500 cases have been published in the scientific literature (see section 7.1).

HICC was recognised as an allergen in 1995 (70) and later included in the new perfume mixture, Fragrance Mix II (71), which is routinely used for the diagnosis of perfume allergy, see above. Furthermore, it is recommended to test separately with HICC, because it is a very frequent allergen (37) and detects relevant fragrance sensitisation which would otherwise have been missed (49). In the studies performed in European dermatology clinics, 0.5-2.7% of eczema patients have been found to be allergic to HICC with the highest frequency in central Europe (55). For further details see Table 4-6.

Table 4-6: Results with fragrance contact allergy screening agents reported since 1999 in patients patch tested for suspected allergic contact dermatitis: **HICC** (5% pet. if not stated otherwise). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS (§).

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI)
Lithuania (72)	Consecutive patients	04/2006-10/2008	816	0.9 (0.3–1.8)§
Spain (49)	Consecutive patients	10/2005-06/2008	852	0.8 (0.3–1.7)§
Germany (CH, AT) (73)	Consecutive patients	03/2000-02/2001	3245	1.9 (1.5–2.4)§

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI)
Germany (CH, AT) (74)	Consecutive patients	01/2003-12/2004	21325	2.4 (2.2–2.6)§
Germany (CH, AT) (68)	Consecutive patients	01/2005-12/2008	35582	2.3 (2.2–2.5)§
Belgium (47)	Consecutive patients	2002-2005	2901	2.1 (1.6–2.7)§
Denmark (69)	Consecutive patients	2005-2008	12302	2.4 (2.1–2.7)§
South Korea (56)	Consecutive patients	04/2002–06/2003	422	1.7 (0.6–3.4)§
USA, Canada (64)	Probably consecutive patients	2003	1603	0.4 (0.2–0.9)§

Myroxylon pereirae (Balsam of Peru)

Myroxylon pereirae is a balm obtained from a Central American tree. It is used as a screening substance for fragrance allergy in Europe and other geographical areas. Although the crude balm is not used in Europe in cosmetics, extracts and distillates are used (75). This natural mixture has been employed as screening agent in the baseline series for many decades. Hence, a wealth of data is available; Table 4-7 summarises results of the past 10 years.

Table 4-7: Results with fragrance contact allergy screening agents reported since 1999 in patients patch tested for suspected allergic contact dermatitis: ***Myroxylon pereirae* resin** (Balsam of Peru) (25% pet.). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS (§).

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI)§
Tel Aviv, Israel (59) #	Consecutive patients	1999-2000	943	6.6 (5.1–8.4)§
South Korea (56)	Consecutive patients	04/2002 – 06/2003	422	7.3 (5.1–10.3)§
Tel Aviv, Israel (60)	Consecutive patients	1998-2004	2156	3.6 (2.9–4.5)§
Manipal, India (58)	Dermatitis patients	1989-1998	1780	1.0 (0.5 – 1.5) §
Tehran, Iran (61)	Consecutive patients	2002-2004	250	2.4 (0.9–5.2)§
Sevilla, Spain (76)	Consecutive patients	2002-2004	863	5.8 (4.3–7.6)§
Ankara, Turkey (62)	Consecutive patients	1992-2004	1038	2.1 (1.3–3.2)§
Vienna, Austria (16)	Consecutive patients of one clinic	1997-2000	2660	5.4 (4.6–6.3)§
Czech Republic (40)	Consecutive patients	1997-2001	12058	7.3 (6.8–7.8)§
Spain (49)	Consecutive patients	10/2005-06/2008	1253	6.4 (5.1–7.9)§

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI)[§]
Copenhagen, Denmark (52)	Consecutive patients	1985-2007	16173	3.9 (3.6–4.2)[§]
Sweden (38)	Consecutive patients	2000	3790	6.5
Nine European countries (53)	Consecutive patients	2002-2003	9672	6.1
Germany, three Swiss and one Austrian Dept. (43)	Consecutive patients	2005-2008	36919	8.0 (7.7–8.3)
Ten depts. From seven EU countries (66)	Consecutive patients	1996-2000	26210	6.0
USA (Canada) (64)	Probably consecutive patients	2003	1603	6.6
NACDG 2009 (65)	Consecutive patients	2005-2006	4449	11.9

Oil of turpentine

This natural extract is not tested in all baseline series. It is considered as a minor screening allergen for fragrance contact allergy. Moreover, oil of turpentine is used as a raw material in perfumery (see Annex I). Table 4-8 summarises results of the past 10 years with patch testing of consecutive patients.

Table 4-8: Results with fragrance contact allergy screening agents reported since 1999 in patients patch tested for suspected allergic contact dermatitis: **Oil of turpentine** (10% pet.) patients patch tested for suspected allergic contact dermatitis. If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS ([§]).

Country	Population	Year(s)	No. tested	Crude % positive (95% CI)[§]
Lisbon, Portugal (77); virtually no .delta.-3-carene	Consecutive patients	1979-1983	4316	2.3 (1.9–2.8)[§]
Birmingham, UK (78)	Potters with occup. hand dermatitis	6 months; prior to 1996	24	14/4 pos. to "Indonesian turpentine"
Austria/Germany (IVDK) (79)	Consecutive patients	1992-1995	27658	0.47 (0.39–0.55)[§]
Austria/Germany (IVDK) (42)	Consecutive patients	1996-2002	59478	Annual prevalence 1.6 to 4.4%
Augsburg, Germany (80)	Population sample	1998	1141	1.2% (on population level!)
Europe (ESSCA) (53)	Consecutive patients	2002/03	3767	1.6%
Austria/Germany/ Switzerland (IVDK) (43)	Consecutive patients	2005-2008	37163	1.8%

An “overall burden” of fragrance contact allergy, in terms of the prevalence of contact allergy to at least one of the up-to-five screening allergens present in the baseline series (FM I, FM II, HICC, *Myroxylon pereirae*, oil of turpentine) has not been given in the published studies. A re-analysis of data from the two published studies of the IVDK (43, 68), covering central Europe from 2005 to 2008 (Germany, Austria and Switzerland), yielded an estimate of such overall prevalence of 16.2% (95% CI: 15.8-16.6%) (IVDK technical report, 2011-11-18).

4.3.3. Population-based epidemiology

In principle, the examination of a representative sample of the population is the most valid approach for estimating disease frequency, as there is no systematic selection process. However, in practice, participation of much less than 70% of those approached introduces the possibility of self-selection and thus of biased morbidity (or risk) estimates. Moreover, the resources needed prohibit regular, e.g. yearly, patch test studies in a sample of several thousand persons. For these reasons few studies exist (see Table 4-9).

A Swedish study of hand eczema in an industrial city showed that among 1,087 individuals recruited from the general population with symptoms of present or previous hand eczema, 5.8% were positive to the Fragrance Mix (81). In Denmark, Fragrance Mix sensitivity was found in 1.1% (0.3-2.1%) of 567 persons drawn as a sample from the general Danish population; only nickel sensitivity was more prevalent (82). In Italy, female patients with hand eczema caused by contact with detergents were patch tested. Of 1100 women, 3.1% reacted to Fragrance Mix I (83). A control group of 619 female patients with no eczema disease were also patch tested; 1.3% were positive to the Fragrance Mix (83). On the other hand, in a sample of 593 healthy Italian recruits, only three positive reactions (0.50%) to FM I were observed (84). Among Danish school children, 14-15 years of age, fragrance contact allergy was detected in 1.8% by patch testing with Fragrance Mix I (85). A study of 85 American student nurses showed that 15 (17.6%) had a positive reaction to Fragrance Mix I; 12 of the individuals also had a positive history of contact dermatitis (86). In this study the concentration of Fragrance Mix I was 16% as opposed to the currently recommended concentration of 8% and the study included only young females. Both of these factors may have contributed to the high prevalence of fragrance sensitivity found.

In 1990, 1998 and 2006, samples of the Danish adult population living in the Copenhagen area were patch tested with the European baseline series. In total 4299 individuals aged 18-69 years (18-41 years only in 1998) completed a pre-mailed questionnaire and were patch tested with FM I and *Myroxylon pereirae* (82, 87, 88). In 1990, 1.1% were found positive to FM I and in 2006, 1.6% were positive, which means no general change. However, when the age group of 18-41 years was analysed, the prevalence of FM I sensitisation followed an inverted V-pattern among women, i.e. an increase from 0.7% in 1990 to 3.9% in 1998, followed by a decrease to 2.3% in 2006. The participation rate varied in the three samples from 71.5% in 1990 to 52.4% in 1998, and to 43.7% in 2006 (82, 87, 88).

Contact sensitisation to FM I is strongly age related, with the relative risk more than doubling in the older age groups, compared to younger PT patients. This has been found in both bivariate (89) and adjusted multifactorial analyses (90). Hence, in older samples of the population, the prevalence of contact allergy to fragrance ingredients in general, and to FM I in particular, can be expected to be higher than in younger samples. From this background, the strikingly high prevalence observed in the MONICA/KORA allergy study in Augsburg, Germany (see Table 4-9) (80), may be explained, together with some residual confounding from the rather complex sampling process.

Table 4-9: Results from patch testing with Fragrance Mix I in different population based groups.

Country (Ref.)	Population	Year(s)	No. tested	% positive (95% CI)
Italy (83)	Females without eczema	Not given	619	1.3
Italy (84)	Male recruits	Not given	593	0.50
Denmark (82)	Population sample adults,15-69 years	1990-91	567	1.1
Denmark (85)	School children 12-16 years old	1995/96	717	1.8
Denmark (82, 87)	Population sample adults, 18-41 years	Jan-Nov 1998	414	2.7
Denmark (88)	Population sample adults, 18-69 years	June 2006–May 2008	3460	1.6
Norway (91)	Population sample adults,18-69 years. (Results reported in 2007)	1994 (92)	1236	1.8 (1.1–2.7)
Germany (80)	Subgroup of MONICA sample, age 25-74	1994/95	1141	11.4
USA (86)	Student nurses, females	1980	85	17.6*
Sweden (81)	Population sampleadults, age 20-65 years reporting hand eczema	1983-84	1087	5.8*

Note: * Testing performed with Fragrance Mix I, containing 16% allergens; the currently used Fragrance Mix I contains 8% allergens (see above).

Table 4-10: Results from patch testing with other fragrance allergens in different population based groups. If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS (§).

Country (Ref.)	Population	Year(s)	Fragrance allergen	No. tested	% positive (95% CI)§
Thailand (93)	Convenience sample (via advertisement), age 18-55	Not given	Isoeugenol, *Evernia prunastri*, *Myroxylon pereirae* *	2545	Positive to at least one of three allergens: 2.5 (1.9–3.2)§
Germany (80)	Subgroup of MONICA sample, age 25-74	1994/95	*Myroxylon pereirae*	1141	2.4
Denmark (88)	Population sample, age 18-69	1990 2006	*Myroxylon pereirae*	567 3460	1.1 0.1

Note: * *Myroxylon pereirae* is a balm obtained from a Central American tree. It is used as a screening substance for fragrance allergy in Europe and other geographical areas. Although the crude balm is not used in Europe in cosmetics, extracts and distillates are used (75).

4.4. Consumer products as a cause of fragrance contact sensitisation and allergic contact dermatitis

4.4.1. Clinical relevance

Clinical relevance is a concept used to describe the significance of a positive (allergic) patch test reaction for an individual patient: a reaction is deemed relevant if contact allergy to the substance is associated with previous or current episodes of allergic contact dermatitis. Thereby, the evaluation of clincial relevance links past exposure to morbidity. For the evaluation of relevance, past or recent exposure(s) to the allergen need to be identified in the patient's history. The success of this process generally depends on:

- The patient's understanding and awareness;
- The dermatologist's knowledge concerning exposures;
- Ingredient labelling; and
- Information about the actual chemical composition of the implicated product.

As these requirements may be met to a varying extent, the validity of relevance information as reported in clinical studies may also be variable. However, information on clinical relevance is important, in principle, because the proportion of currently relevant sensitisations reflects the amount of current exposure and resulting disease state, which may increase or decrease with time. In this way, current relevance also reflects the direct burden of a fragrance contact allergy to the individual and indirectly to society. Further important aspects of the evaluation of clinical relevance as a final step of patch testing have been discussed (32, 94-96).

Generally, clinical relevance is categorised as “current”, “previous” or “unknown”. Further differentiation has been introduced by adding information on:

- Occupational versus non-occupational causation; and
- The level of certainty of the relevance statement, e.g. as “certain”, “probable”, “possible”.

In some cases, clinical relevance may not be established due to:

- Immunological cross-reactivity with an individual allergen, diagnosed or not;
- Active sensitisation by the patch testing;
- Contact sensitisation not caused by the substance, but by a contaminating constituent; or
- Failure to test with a true hapten (e.g. haptens formed from prehaptens on exposure to air, see chapter 5).

It should be noted that this statement on clinical relevance refers to the past history of a patient. This implies that a lack of, or unknown, clinical relevance does not make future allergen avoidance unnecessary.

In the context of contact allergy to fragrance ingredients, a number of alternative concepts of relevance have been used, for example:

- A history of intolerance to perfume or to perfumed products;
- A history of intolerance to perfume actually containing the allergen diagnosed;
- Detection of the culprit allergen in a perfume previously used.

4.4.2. Elicitation with clinical symptoms/signs, current and past

In case reports or small series, the clinical relevance of positive patch test reactions is usually well established and presented in detail. Moreover, a few large-scale clinical studies on contact allergy to fragrance ingredients have reported results on clinical relevance, which will be presented and discussed in this section. The studies can be subdivided into those which focus on medical history, patch testing with consumer products or detection of specific allergens in consumer products used by patients.

Medical history

A series of studies conducted in the 1990s showed that most individuals with contact allergy to fragrance ingredients were aware that they could not tolerate fragranced products on their skin and were able to specifically name product categories that initiated their disease (9). In this context, colognes, deodorants and lotions were named significantly more often by fragrance allergic dermatitis patients than by patients without fragrance contact allergy (3). These studies are described in the SCCNFP opinion on fragrance allergy of 1999 (1). Newer studies are outlined below.

NACDG 2009 study (65)

The definition of "present" clinical relevance in this North American network study was strict, requiring:

- A positive use or patch test with the suspected item(s) for "definite" relevance; and
- Verification of the presence of the allergen in known skin contactants, and consistent clinical presentation for "probable".

If these conditions were not met, but skin contact to items generally containing the item was likely, "possible" was used.

Regarding fragrance allergens, the proportions were as described in Table 4-11.

Table 4-11: Extract from ((65) Table 3) regarding the proportion of patients with "present clinical relevance" (see text) and "past clinical relevance" (criteria not given).

Fragrance allergen	n (tested)	% (pos.)	Current relevance (%)			Past relevance (%)
			Definite	Probable	Possible	
Myroxylon pereirae	4449	11.9	1.3	33	53	2.7
FM I	4439	11.5	2.0	29.4	54.3	4.3
Cinnamal	4435	3.1	1.5	33.8	50	2.9
Ylang-Ylang oil	4434	1.5	4.6	10.8	73.8	1.5
Jasmine absolute	4447	1.1	0	24.5	67.3	6.1

Frosch 2002 (a) study (67)

In this study, 1,855 consecutive patients were patch tested with FM I and a series of a further 14 fragrance chemicals. Prior to the test, the history of adverse reactions to fragrances was classified as "certain" (6.6%), "probable" (8.0%), "questionable" (9.2%) or "none" (76.1%) (see (71)).

Frosch 2002 (b) study (97)

A series of 18 essential oils or components thereof, together with FM I, was assessed in 1,606 consecutive patients. Similar to the above study, the proportions of patients with a "certain" or "probable" history (or otherwise) and positive reactions to either FM I or the

special series, or both, were cross-tabulated. Of note, 53.7% of patients with positive reactions to FM I only, had no history. Similarly 54.2% of patients with positive reactions only to one of the essential oils had no history. However, in cases of reactivity to both FM I and one of the essential oils, the proportion of patients with no history was only 36.5%.

Frosch 2005 study (35)

The diagnostic properties of FM I and the new FM II were evaluated in 1,701 consecutive patients patch tested in six European centres. Contrasting a "certain" (found in 8.7% of patients) with "no history" (75.3% of patients), the sensitivity of FM I was 25.2%, and the positive predictive value (PPV) 45.1%. In comparison, the sensitivity of FM II at 14% concentration was 13.5% and the PPV was 55.6%. The combination of the two mixes was important, as more patients with a "certain" history, but also independently from history, reacted to just one of the mixes rather than to both.

Danish Contact Dermatitis Group 2005-2008 (69)

In 12302 consecutive patients patch tested in seven dermatology clinics and three university hospitals, 10.6% were positive to one or more of the fragrance allergy markers (FM I, FM II, *Myroxylon pereirae* or hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)). Clinical relevance covered current and/or past relevance based on: 1) medical history; 2) results of patch and/or use tests; 3) ingredient labelling: or 4) chemical analysis. Clinical relevance was found in 71.0% of cases positive to FM I, 72.2% of those positive to FM II and 76.7% of those positive to HICC. These proportions were higher than the average for other cosmetic allergens such as preservatives and hair dyes, which gave relevant reactions in about 50% of those positive, as did *Myroxylon pereirae. Myroxylon pereirae* itself is not used in cosmetics as it is banned, but sensitisation may be caused by exposures to related substances and thus relevance may be difficult to determine.

Cosmetic products

Fragrance formulae from cosmetic products

Popular fine fragrances (5), as well as toilet soaps, shampoos, lotions, deodorants, and aftershaves have been shown to provoke allergic contact dermatitis in patients when used for patch testing (5, 6, 98, 99). Moreover, commercially available fragrance formulae and dilutions of individual fragrance allergens were potent elicitors of allergic contact dermatitis under simulated use conditions (10, 100, 101).

More recently, deodorants spiked with the fragrance allergens cinnamal, hydroxycitronellal and HICC, respectively, in realistic in-use concentrations were shown to elicit allergic contact dermatitis in 89-100% of the fragrance allergic individuals tested (102-104). In 87.5% of HICC sensitised individuals the use of a cream (and in 82.8% the use of an ethanol solution) spiked with HICC provoked dermatitis (105). These studies are discussed in more detail in chapter 11 on quantitative aspects. Other new studies are mentioned below:

IVDK "own perfumes" study (106)

A different perspective on clinical relevance is provided by assessing the proportion of positive reactions to the FM I or single fragrance allergens in patients who had not tolerated certain perfumed products, such as deodorants and aftershaves and who were patch test positive to these cosmetics. The following two tables are taken from this publication.

Table 4-12: Extract from ((106) Table 2) on the frequency of positive reactions to fragrance allergens in patients with vs. without positive patch test reaction to their own deodorant.

Fragrance allergen	Conc. (%)	Deodorant positive (n=66)		Deodorant negative (n=855)	
		n (test)	% pos. (95% CI)	n (test)	% pos. (95% CI)
Fragrance Mix I	8	61	38.0 (24.1-51.9)	805	15.0 (12.5-17.5)
Myroxylon pereirae	25	60	22.9 (12.7-33.1)	806	9.1 (7.2-11.0)
Hydroxycitronellal	1	33	6.5 (0.7-12.3)	204	4.3 (1.5-7.1)
Isoeugenol	1	33	6.5 (0.7-12.3)	204	7.2 (3.6-10.8)
Cinnamal	1	29	11.3 (0-24.1)	133	1.1 (0-2.7)
Geraniol	1	29	8.3 (0-20.4)	141	0 (0-2.1)

Of the 66 patients with a positive patch test reaction to their own deodorant, most had positive reactions to one or more fragrance allergens. This was much more prevalent than those patients in whom no positive reaction to their deodorant was observed. This observation supports the notion that the respective fragrance allergens are important in contact allergy to fragrance ingredients caused by deodorants, supporting data regarding exposure (chapter 10.1).

Table 4-13: Extract from ((106) Table 2) on the frequency of positive reactions to fragrance allergens in patients with vs. without positive patch test reaction to their own aftershave, eau de toilette or perfume.

Fragrance allergen	Conc. (%)	Product positive (n=63)		Product negative (n=819)	
		n (test)	% pos. (95% CI)	n (test)	% pos. (95% CI)
Fragrance Mix I	8	56	57.1 (46.2-68.1)	764	13.9 (11.4-16.4)
Myroxylon pereirae	25	56	13.9 (7.3-20.4)	766	8.8 (6.8-10.7)
HICC	5	20	58.3 (37.5-79.0)	310	1.3 (0-2.7)
Evernia prunastri	1	28	22.1 (7.0-37.2)	153	8.8 (4.2-13.4)
Hydroxycitronellal	1	33	6.5 (0.7-12.3)	204	4.3 (1.5-7.1)
Cananga odorata (ylang-ylang oil)	10	7	16.3 (2.0-30.5)	43	5.0 (0-11.3)

Similar results were obtained from the subgroup of patients with a positive reaction to their eau de toilette, aftershave (hydroalcohol solutions) or perfumes (Table 4-13). However, notable differences were: (i) the greater relative importance of *Evernia prunastri* (Oak moss absolute); and (ii) generally an extremely high proportion of positive reactions to various other fragrance ingredients.

4.4.3. Elicitation in diagnostic patch tests without clinical history

In a variable proportion of patients, a positive patch test reaction does not correlate with recent or past episodes of presumptive allergic contact dermatitis. Apart from particular circumstances, such as cross-reactivity or reactivity to contaminants outlined above, there are several possible explanations for this:

- The patch test reaction was a false-positive (irritant).
- There was erroneous recall/interpretation of the patient's history (false-negative).
- Lack of knowledge concerning exposures.

- If the patient is weakly sensitised (e.g. by a low induction dose), the occlusive exposure during patch testing may have been the only exposure above the individual elicitation threshold capable of eliciting an unequivocal allergic contact reaction. In this situation, clinical relevance would be classified as "unknown". Nevertheless, there is an alteration of the immune status of the individual.

Sometimes, a repeated open application or provocative use test is employed to mimic "normal" exposure to the allergen. A positive reaction to such a use-related test confirms actual sensitisation. Moreover, the positive result supports the necessity of future allergen avoidance. Apart from the risk of developing allergic contact dermatitis in the future, sensitisation means an alteration of the immune status of the individual.

4.5. Socio-economic impact of contact allergy

4.5.1. Health related quality of life

Skin diseases in general are known to affect quality of life significantly (107); this also applies to eczema, where most studies concern atopic dermatitis and hand eczema patients (108, 109). Hand eczema has a poor prognosis and may affect the self-image, limit social activities and lead to occupational restrictions (109, 110). The quality of life in hand eczema patients with fragrance contact allergy is affected in a similar degree as patients with other contact allergies (111).

In a questionnaire study of 117 patients recently diagnosed with contact allergy to fragrance ingredients, most presented with hand or facial eczema. In response to the question if and how fragrance allergy had affected their life situation, 67.5% replied that they often had to take special precautions, 47.0% replied that they were often bothered by eczema and itch, 17.1% said that they had had to take sick leave due to their fragrance contact allergy and 45.3% felt that fragrance contact allergy had significantly influenced their daily living (112).

4.5.2. Occupational restrictions

Contact allergy is known to influence severity and prognosis of hand eczema (113, 114) including risk of sick leave (111). Fragrance contact allergy is mostly of a non-occupational origin (90) related to the personal use of scented cosmetics, but may have secondary occupational consequences. This may be due to exposure to fragrance ingredients also in the work place or because hand eczema has developed. Hand eczema itself may make it impossible to remain in the trade even if protective equipment is used. In young people, fragrance allergy may limit the choice of occupations, as it will be difficult to work as a hairdresser, cosmetologist or in other occupations with a significant skin exposure to fragranced products.

4.5.3. Costs to health care/health economics

In a population based study of 3,460 individuals, contact allergy to FM I was found in 1.6%; logistic regression analyses showed that medical consultation due to cosmetic dermatitis (OR 3.37, 95% CI 1.83-6.20) and cosmetic dermatitis within the past 12 months (OR 3.53, CI 2.02-6.17) were significantly associated with sensitisation to FM I (88). Further, as mentioned above, fragrance allergy may lead to sick leave (112). No specific cost estimates for fragrance allergy exist, but the yearly total costs of contact dermatitis in Western Europe was estimated to be 5.2 billion Euro in 1997. Prices were based on the Allergy White Paper (1997) and on results of investigations and extrapolations of known data for Western Europe (115). Fragrance allergy is the second most frequent cause of contact allergy after nickel allergy and is seen in every 10th patient investigated for contact allergy. Even a modest reduction in nickel allergy has been estimated to have the value of 12 million Euro/year/million people in Denmark (Environmental Project Nr. 929, 2004; http://www2.mst.dk/Udgiv/publications/2004/87-7614-295-7/pdf/87-7614-296-5.pdf, last

accessed 2011-11-13). The costs are likely to differ in other countries, some with higher expenses and some with lower costs. These estimates show that the cost of contact allergy in the population may be considerable.

4.6. Allergen avoidance

Generally, "allergen avoidance" can be regarded as having two aspects: (i) primary prevention of the acquisition of contact allergy achieved by avoiding or limiting exposure of the general population, or certain parts of it, to allergens; and (ii) secondary prevention in terms of avoiding (re-)elicitation of allergic contact dermatitis in sensitised individuals.

4.6.1. Primary prevention: limiting or eliminating exposure to allergens in the population

The main aim of public health is the primary prevention of disease in populations. Allergic contact dermatitis (to fragrances) has the potential to have a significant impact on quality of life, including effects on fitness for work (chapter 4.5). Moreover, it is a common phenomenon and therefore a reduction of exposure to (fragrance) allergens must be an objective of effective Public Health measures.

Means of limiting or eliminating exposure to fragrance allergens include the following:

- *Prohibition* by regulatory measures or other means.
- *Restriction* by regulatory measures or other means of the maximum permissible concentration of a substance, or a critical component of natural mixtures, possibly according to different uses and product types, respectively.
- *Substitution* with suitable, but less or non-allergenic compounds. Substitution by a component which is chemically different, but effectively not different in terms of allergenicity or cross-reactivity, is not adequate (e.g. an ester) (chapter 5).
- *Formulating the fragrance* with the aim of limiting or eliminating those substances for which a sensitising potential has been shown. One difficulty with this approach is that sometimes no sensitisation data exist for those components of a fragrance formula which are used to replace a "known sensitiser".
- *Deliberate avoidance* of the use of fragrances where they are not essential to the function of a finished product, but used merely to add to its appeal. Examples could include most cosmetics, topical medicaments, detergents etc., but obviously not perfumes, eau de toilette and other products used for their scent.
- *Information, e.g. labelling* so that the consumer may make an informed choice to avoid exposure to a particular ingredient.

4.6.2. Secondary prevention: avoiding re-exposure to (a) specific sensitiser(s) in clinically diagnosed individuals

In clinical dermatology, avoidance of re-exposure to an allergen is central to the care of sensitised patients. Contact sensitisation, as a latent condition, persists life-long, and therefore allergen avoidance is the only means of avoiding potentially severe and/or handicapping disease, which affects quality of life and may affect fitness for work, i.e. allergic contact dermatitis.

In this context, the valid diagnosis of sensitisation, by patch testing (32) with standardised materials, is a prerequisite of successful allergen avoidance.

In the case of fragrances, a history clearly indicative of "fragrance dermatitis" but in which patch testing with commercially available test preparations is negative, most probably reflects a shortcoming of the patch test procedure, namely, a false-negative investigation. An important cause is inadequate information on the presence of fragrance substances

present in cosmetic products (and consumer products in general). This means that patients cannot be tested for relevant substances.

A false-negative investigation can also be due to a number of other reasons: (i) non-adherence to scientific recommendations (32) or guidelines (e.g. (116)); (ii) sub-optimal patch test concentration; or (iii) use of non-oxidised material if oxidised material is the true allergen.

In an “ideal” case, from the point of view of successful patient management, the test procedure identifies all the allergen(s) to which the patient has developed contact allergy, according to the information on the culprit product(s) brought in by the patient. Such contact sensitisation is termed “clinically relevant” (65), and the need for allergen avoidance in the future is unequivocally evident in these cases. However, not infrequently, clinical relevance of an allergic patch test reaction cannot be ascertained for various reasons, which may be beyond control by the clinician (see chapter 4.4). Nevertheless, future elicitation of allergic contact dermatitis by sufficient contact with the identified “non-relevant” allergen may be expected. Hence, the patient will need to avoid the respective substance(s).

In a less “ideal” case, only part of the fragrance allergens having caused allergic contact dermatitis are identified (and can subsequently be avoided), while another part remains unidentified, for instance because it is: (i) not labelled on the product; and/or (ii) not available for routine diagnostic patch testing (special investigations such as chemical analysis of the culprit product, and break-down patch testing of its individual components, are performed rarely). Such “residual” undetermined sensitisation will hamper the success of secondary prevention of allergic contact dermatitis due to fragrances.

The above consideration raises the question for the patient of how to identify fragrance chemicals in cosmetics and other products coming into contact with the skin, such as detergents and household products, topical medicaments, products used professionally (e.g. by hairdressers, beauticians, masseurs, aromatherapists), and in other industrially used categories of products (7) (see also chapter 9). In this regard, the labelling with “perfume” or “contains fragrances” does not provide sufficient information. Moreover, such general labelling has two main disadvantages:

- It does not aid the identification of past exposure to specific agents when planning a patch test and later, when interpreting possible positive patch test results regarding clinical relevance.
- The diagnosis of allergic contact sensitisation to unidentified fragrance allergens will lead to unnecessary avoidance of other fragrance substances to which the patient is not sensitised, which are, however, included under the label “perfume”.

Furthermore, the attribute “fragrance-free” may be misleading, as it merely states that no substance was added to the product to give it a scent, assuming it is used correctly at all. Nevertheless, fragrance substances used for other purposes, e.g. as preservatives, may expose the “fragrance allergic” patient to the allergen even in a “fragrance free” product (117). However, in terms of cosmetic ingredient labelling, such other uses are less problematic, as each ingredient not used as a fragrance component must be labelled. Also the use of natural products (essential oils) as preservatives must be considered in this context.

Ingredient labelling of 26 individual fragrance ingredients, identified as allergens in humans, was introduced for cosmetics in 2005. The intention was to provide a tool for clinicians for optimizing the investigation of patients with suspected fragrance allergy, as well as for fragrance allergic patients for avoiding products containing substances they have been shown to be allergic to. Both these aims are objectives of secondary prevention and seem to have been well accepted. In a study of fragrance allergic patients and their utilisation of ingredient labelling (112), most responded that they used the ingredient labelling (86.3%) and of those who used it, the majority (65.3%) found it helpful (112). Most allergic patients used the ingredient labelling (83.2%) to find out if the product was scented, while 35.6%

also looked for specific ingredients. Many (84.9%) found that a clearer labelling, e.g. easier names and a larger font size, would increase their benefit.

4.7. Conclusions

Contact allergy to fragrances is relatively common, affecting 1 to 3% of the general population, based on limited testing with eight common fragrance allergens and about 16 % of patients patch tested for suspected allergic contact dermatitis. Fragrance contact allergy is mostly non-occupational and related to the personal use of cosmetic products.

Allergic contact dermatitis can be severe and widespread, with a significant impairment of quality of life and potential consequences for fitness for work. Thus, prevention of contact sensitisation to fragrances, both in terms of primary prevention (avoiding sensitisation) and secondary prevention (avoiding relapses of allergic contact dermatitis in those already sensitised), is an important objective of public health risk management measures.

5. Activation of weak or non-sensitising substances into sensitisers - prehaptens and prohaptens

Fragrance allergens act as haptens, i.e. low molecular weight chemicals that are immunogenic only when attached to a carrier protein. However, not all sensitising fragrance chemicals are directly reactive, but require previous activation.

A prehapten is a chemical that itself is non- or low-sensitising, but that is transformed into a hapten outside the skin by simple chemical transformation (air oxidation, photoactivation) and without the requirement of specific enzymatic systems.

A prohapten is a chemical that itself is non- or low-sensitising but that is transformed into a hapten in the skin (bioactivation) usually via enzyme catalysis.

It is not always possible to know whether a particular allergen that is not directly reactive acts as a prehapten or as a prohapten, or both, because air oxidation and bioactivation can often give the same product (geraniol is an example).

Some chemicals might act by all three pathways. One example is geranial (an isomer of citral) which is a hapten itself with a moderate sensitisation potency, but can be activated to more potent sensitisers via air oxidation (autoxidation) thus acting as a prehapten and also via bioactivation (metabolic activation) thus acting as a prohapten (118).

Increased understanding of the importance of activation through interaction with the environment that turns non-sensitising compounds into sensitisers has made it important to distinguish between prehaptens and prohaptens. This distinction facilitates discussions by emphasizing the differences in activation mechanisms between the two types of compounds requiring activation to become haptens. It is important to note that prehapten activation, in contrast to bioactivation, can be prevented to a certain extent by avoidance of air exposure during the handling and storage of the chemicals. This concerns the most prominent haptens formed by autoxidation i.e. the hydroperoxides. In bioactivation, hydroperoxides have not been identified as metabolites, but other allergenic oxidation products (in particular aldehydes and epoxides) have been identified as being formed by both activation routes depending on the structure of the compound. One thoroughly studied example is geraniol which forms the aldehyde geranial, epoxy-geraniol, and also epoxy-geranial via both pathways of activation (autoxidation and metabolic oxidation) (119, 120). When haptens are formed by both pathways, the impact on the sensitisation potency depends on the degree of autoxidation in relation to the amount of metabolic oxidation.

Human data on established prehaptens are presented in Table 5-1 and Table 5-2. In Table 5-1 the results from patch testing with air exposed samples of the prehaptens are given. Table 5-2 shows the results from testing with the prehaptens themselves without intended air exposure. In addition to the data given in this chapter, animal data (LLNA) on the pure prehaptens or after controlled air exposure are given in Table 8-2. Possible pro- and prehaptens are identified by SAR analyses in chapter 9.

5.1. Prehaptens

Autoxidation is a free radical chain reaction in which hydrogen atom abstraction in combination with addition of oxygen forms peroxyl radicals. The reaction shows selectivity for positions where stable radicals can be formed. So far, all fragrance substances that have been investigated with regard to the influence of autoxidation on the allergenic potential, including identification of formed oxidation products, have oxidisable allylic positions that are able to form hydroperoxides and/or hydrogen peroxide as primary oxidation products upon air exposure. Once the hydroperoxides have been formed outside the skin they form specific antigens and act as skin sensitisers (121). Secondary oxidation products such as aldehydes and epoxides can also be allergenic, thus further increasing the sensitisation potency of the autoxidation mixture (122). The process of photoactivation may also play a role, but further research is required to establish whether this activation route is currently underestimated in importance due to insufficient knowledge of the true haptens in this context.

Most terpenes with oxidisable allylic positions can be expected to autoxidise on air exposure due to their inherent properties. Depending on the stability of the oxidation products that are formed, a difference in the sensitisation potency of the oxidised terpenes can be seen. Oxidation products of commonly used fragrance terpenes (limonene, linalool, geraniol, linalyl acetate) have been identified as potent sensitisers in predictive animal tests (119, 123-128) (see chapter 8). This is also demonstrated for alpha-terpinene (129) and citronellol (AT Karlberg, personal communication 2012). The oxidised fragrance terpenes limonene, linalool and linalyl acetate have been tested in consecutive dermatitis patients and give frequent allergic contact reactions (130-135). Not all oxidised fragrance substances are strong sensitisers, e.g. caryophyllene is readily oxidised but has a low sensitisation potency after autoxidation (136). This is supported by clinical studies showing oxidised caryophyllene to be a less frequent allergen compared to oxidised limonene and oxidised linalool (133). Details are given in Table 5-1 The non-oxidised compounds rarely cause allergic reactions (43-45, 67, 70, 74, 97, 137-139), for details see Table 5-2. As oxidised and non-oxidised fragrance terpenes were not patch tested simultaneously in the same patients, the results are presented in two separate tables (Table 5-1 and Table 5-2).

Oxidised fragrance terpenes with defined content of the major haptens formed after autoxidation have not been commercially available for testing in dermatology clinics. In the published clinical studies testing oxidised fragrence terpenes, the patch test preparations have been obtained specifically for the performed multicentre studies. From 2012, patch test preparations of oxidised limonene and oxidised linalool with defined content of the major allergens in the oxidation mixtures, i.e. the hydroperoxides, are commercially available.

It should be noted that activation of substances via air oxidation results in various haptens that might be the same or cross-reacting with other haptens (allergens). The main allergens after air oxidation of linalool and linalyl acetate are the hydroperoxides. If linalyl acetate is chemically hydrolysed outside the skin it can thereafter be oxidised to the same haptens as seen for linalool. A corresponding example is citronellol and citronellyl acetate. In clincal studies, concomitant reactions to oxidised linalool and oxidised linalyl acetate have been observed (140, 141). Whether these reactions depend on cross-reactivity or are due to exposure to both fragrance substances cannot be elucidated as both have an allergenic effect themselves.

Linalool and linalyl acetate are the main components of lavender oil. They autoxidise on air exposure also when present in the essential oil, and form the same oxidation products found in previous studies of the pure synthetic terpenes. Experimental sensitisation studies showed that air exposure of lavender oil increased the sensitisation potency. Patch test results in dermatitis patients showed a connection between positive reactions to oxidised linalool, linalyl acetate and lavender oil (140).

Air oxidation of prehaptens can be prevented to a certain extent by measures during handling and storage of the ingredients and final products to avoid air exposure, and/or by addition of suitable antioxidants. The autoxidation rate depends not only on the compound itself, but also on its purity. The prevention of autoxidation using antioxidants needs thorough investigation because antioxidants can exert their function by being oxidised instead of the compound that they protect and might thereby be activated to skin sensitising derivatives after oxidation, which is the case for alpha-terpinene from tea tree oil (129). Alpha-Terpinene together with its analogue gamma-terpinene has been suggested as an agent for maintaining the oxidative stability of different matrices, such as food, cosmetics and medicaments (142-144). As antioxidants are now frequently used at elevated concentrations in scented products due to a growing awareness of the problem of autoxidation, there is a risk that sensitisation caused by the antioxidants will rise. One of the most used antioxidants is butylated hydroxytoluene (BHT) which is considered a minimal risk for sensitisation in the concentrations used but nevertheless, with increased concentrations and usage, the risk of sensitisation could increase.

Due to the complexity of scented products, which are mixtures of many different fragrance substances, there are at present no published data identifying the presence of individual hydroperoxides in cosmetic products containing the above fragrance terpenes. However, clinical studies show a clear connection between contact allergy to oxidised limonene and oxidised linalool, and contact allergy to other markers of fragrance contact allergy (130-135); see Table 5-3.

Table 5-1: Contact allergic reactions to the autoxidised fragrance substances limonene, linalool, caryophyllene, myrcene and linalyl acetate in consecutive dermatitis patients.

INCI name	CAS no	Test conc. (%)	n Positive/n tested (%)	Comments (Ref.)
D-Limonene (ox.)	5989-27-5	5	18/703 (2.6%)	§ (130)
		3	28/1172 (1.6%)	
		2	3/362 (0.83%)	
D-Limonene (ox.)	5989-27-5	3	63/2273 (2.8%) variation between centres: 0.3-6.5%	§ (131)
D-Limonene (ox.)	5989-27-5, 5989-54-8, 138-86-3	3	49/1812 (2.3%)	§ (134)
L-Limonene (ox.)			36/1812 (2.0%)	
D – and/or L- Limonene (ox.)			63/2411 (2.6%)	
Linalool (ox.)	78-70-6	2	20/1511 (1.3%) variation between centres: 0.4-2.7%	§ (133)
Caryophyllene (ox.)	88-44-5	3.9	2/1511 (0.1%)	
Myrcene (ox.)	123-35-3	3	1/1511 (0.1%)	
Linalool (ox.)	78-70-6	2	14/1693 (0.83%)	§ (135)
		4	67/2075 (3.2%)	
		6	91/1725 (5.3%)	
		11	72/1004 (7.2%)	
Linalool (ox.)	78-70-6	3	11/483 (2.3%)	(145)
Linalyl acetate (ox.)	115-95-7	6	13/1217 (1.1%)	(141)

Notes: § Bicentric or multicentre studies.
(ox.) Oxidised.

Table 5-2: Contact allergic reactions to limonene, linalool, linalyl acetate and caryophyllene in consecutive dermatitis patient. Please observe that several studies have been performed using the test substances without reporting the autoxidation status but it has been intended to be low. For precise information see the original references.

INCI name	CAS number	Test conc. (%)	n Positive/n tested (%)	Comments (Ref.)
Limonene	138-86-3	2	0/1200	(137)
Limonene			3/2396 (0.1%)	§ (74)
DL-Limonene			11/1241 (0.88%)	§ (43)
Limonene			0/320	(44)
DL-Limonene			3/2396 (0.1%)	§ (74)
Linalool	78-70-6	30	0/179	(139)
		20	3/1825 (0.2%)	§ (45)
		10	2/320 (0.6%)	(44)
		10	4/792 (0.5%)	(138)
		5 and 1	0/100	(70)
Linalool, "stabilised" *		10	7/2401 (0.3%)	§ (74)
		10	2/985 (0.2%)	§ (43)
Linalyl acetate	115-95-7	1, 5	0/100	(70)
		10	4/1855 (0.2%)	§ (67)
beta-Caryophyllene	87-44-5	5	10/1606 (0.6%)	§ (97)

Notes: § Bicentric or multicentre studies.
(ox.) Oxidised.
* Stabilised: according to the manufacturer contained additional substances aimed at limiting oxidation.

Table 5-3: Concomitant reactions to fragrance markers: Fragrance Mix I and II (FM I, FM II), *Myroxylon pereire* (MP) and to colophonium (coloph.) in the baseline series in patients with positive or negative patch test reactions to oxidised fragrance substances.

	Total number of pos. and/or neg. reactions	Pos. to FM I		Pos. to MP		Pos. to coloph.		Ref.
		n	%	n	%	n	%	
Reactions to ox. D- limonene and/or limonene hydroperoxide fraction	Pos.: 49	20	41	12	24	12	24	(130)*
	Neg.: 2751	223	8.1	142	5.2	131	4.8	
Reactions to ox. D- limonene and/or limonene hydroperoxide fraction [a]	Pos.: 60	22	37	11	18	13	22	(132)*
	Neg.: 729	141	19	71	9.7	58	8	

Reactions to ox. D- limonene and/or ox. L-limonene [a]	Pos. to ox. D-limonene: 41	14	34	11	27	11	27	(134)*
	Neg. to ox. D-limonene: 1771	113	6.4	91	5.1	62	3.5	
	Pos. to ox. L-limonene: 36	11	31	12	33	9	25	
	Neg. to ox. L-limonene: 1776	116	6.5	80	4.5	64	3.6	
Reactions to any of ox. linalool, myrcene, caryophyllene	Pos. to any of the tested ox. subst.: 31	12	39	6	31	12	39	(133)*
	Neg. to any of the tested ox. subst: 1480	93	6	63	4	46	3	

		Pos. to FM I		**Pos. to FM II**		**Pos. to MP**		**Pos. to coloph.**		
		n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	
Reactions to ox. linalool	Pos. at test conc. 4%: 30	8	26.7	5	16.7	10	33.3	5	16.7	(135)*
	Pos. at test conc. 6%: 55	12	21.8	8	14.5	11	20	8	14.5	
	Pos. at test conc. 11%: 72	14	19.4	9	12.5	14	19.4	9	12.5	
	Total pos. at any test conc: 75/1004	n.g.		n.g.		n.g		n.g.		
	Total neg. at any test conc: 929/1004	56	6.0	29	3.1	45	4.8	24	2.6	

Notes: * Bicentric or multicentre studies.
n.g. Not given.
(ox.) Oxidised.

5.2. Prohaptens

Compounds that are bioactivated in the skin and thereby form haptens are referred to as prohaptens. The human skin expresses enzyme systems that are able to metabolise xenobiotics (146), modifying their chemical structure to increase hydrophilicity and allow elimination from the body. Xenobiotic metabolism can be divided into two phases: phase I and phase II. Phase I transformations are known as activation or functionalisation reactions, which normally introduce or unmask hydrophilic functional groups. If the metabolites are sufficiently polar at this point they will be eliminated. However, many phase I products have to undergo subsequent phase II transformations, i.e. conjugation to make them sufficiently water soluble to be eliminated. Although the purpose of xenobiotic metabolism is detoxification, it can also convert relatively harmless compounds into reactive species. Cutaneous enzymes that catalyse phase I transformations include the cytochrome P450 mixed-function oxidase system, alcohol and aldehyde dehydrogenases, monoamine oxidases, flavin-containing monooxygenases and hydrolytic enzymes. Acyltransferases, glutathione S-transferases, UDP-glucuronosyltransferases and sulfotransferases are

examples of phase II enzymes that have been shown to be present in human skin (146). These enzymes are known to catalyse both activating and deactivating biotransformations (147), but the influence of the reactions on the allergenic activity of skin sensitisers has not been studied in detail.

Skin sensitising prohaptens can be recognised and grouped into chemical classes based on knowledge of xenobiotic bioactivation reactions, clinical observations and/or *in vivo* and *in vitro* studies of sensitisation potential and chemical reactivity. Few mechanistic investigations of prohaptens have so far been published. Investigations that are important for the bioactivation of fragrance substances are studies on alkenes, e.g. alpha- terpinene (148-150), the allylic primary alcohols geraniol (120) cinnamyl alcohol (151-155), eugenol and isoeugenol (156).

In order to be able to predict the sensitisation potency of prohaptens, steps of bioactivation have to be included in the predictive tests where intrinsic bioactivating systems are lacking. So far, no such predictive non-animal methods have been developed that take account of this.

When bioactivation occurs, the risk of cross-reactivity also needs to be be considered. Cross-reactivity between certain aldehydes and their corresponding alcohols, e.g. cinnamal - cinnamyl alcohol and geranial - geraniol, due to the metabolic oxidation of the alcohols to the aldehydes in the skin is demonstrated (120, 151-155).

When using derivatives of a fragrance substance, it must be taken into account that the derivative could be metabolically transformed in the skin into the parent or cross-reacting compounds. A prominent example of such bioactivation is the hydrolysis of esters by esterases to the corresponding original alcohols. The metabolic product obtained can act as a hapten or a prohapten in exactly the same way as the non-esterified parent compound.

Isoeugenol and its derivatives are an important example for this mechanism from which general conclusions may be drawn. As only the use of isoeugenol in fragranced products needs to be indicated on the ingredients list, the additional exposure to isoeugenol through its derivatives should also be taken into account. In a study it was shown that several EDP/EDT/aftershave lotions contained high levels of isoeugenyl acetate and isoeugenol methyl ether (Table 5-4) (157). Isoeugenyl acetate will be hydrolysed by esterases in the skin to generate isoeugenol. The situation may be similar for eugenyl acetate and geranyl acetate, which might be used in fragrance formulations instead of eugenol and geraniol, respectively. Moreover, such derivatives will contribute to exceeding any established `acceptable dose/area level' of the parent compound, i.e., yield unduly high concentrations on the skin.

Table 5-4: Mean and median content of isoeugenol and its derivatives in the 29 perfume products.

Fragrance compound INCI Name	**Products containing the fragrance**		**Content (ppm)**			
	No.	**%**	**Range**	**Mean**	**SD**	**Median**
Isoeugenol	16	55	27-203	71	54	45
Isoeugenyl acetate	10	34	20-4689	985	1570	166
Isoeugenyl methyl ether	13	45	65-1755	360	442.3	222

5.3. Conclusions

- Many fragrance substances can act as prehaptens or prohaptens, forming allergens which are more potent than the parent substance by abiotic and/or metabolic activation. Activation can thus increase the risk of sensitisation.
- Fragrance substances of clinical importance known to be prehaptens and to form sensitising compounds by air oxidation include limonene, linalool, and linalyl acetate.
- Fragrance substances of clinical importance known to be prohaptens and to form sensitising compounds by metabolic transformation include cinnamyl alcohol, eugenol, isoeugenol and isoeugenol acetate.
- Fragrance substances of clinical importance with published data known to be both prehaptens and prohaptens and to form sensitising compounds by air oxidation (prehaptens) and by metabolic transformation include geraniol and alpha-terpinene.
- A fragrance substance that sensitises without activation, but forms more potent sensitising compounds by air oxidation and also by metabolic transformation is, as one example, geranial (one isomer of citral).
- In the case of prehaptens, it is possible to prevent activation outside the body to a certain extent by different measures, e.g. prevention of air exposure during handling and storage of the ingredients and the final product, and by the addition of suitable antioxidants. When antioxidants are used, care should be taken that they will not be activated themselves and thereby form new sensitisers.

  It should be noted that the possibility to reduce the sensitisation potency by preventing air oxidation is also important for a direct acting hapten or prohapten, if a further activation by air oxidation to more allergenic compounds has been shown.
- In the case of prohaptens, the possibility to become activated is inherent to the molecule and activation cannot be avoided by extrinsic measures. Activation processes increase the risk for cross-reactivity between fragrance substances. Cross-reactivity has been shown for certain alcohols and their corresponding aldehydes, i.e. between geraniol and geranial (citral) and between cinnamyl alcohol and cinnamal.

  Cross-reactivity is also expected between ester derivatives and their parent alcohols, as the esters will be hydrolysed by esterases in the skin. Esters of important contact allergens that can be activated by hydrolysis in the skin are isoeugenol acetate, eugenyl acetate and geranyl acetate all of which are known to be used as fragrance ingredients.
- Further experimental and clinical research in the area of abiotic and/or metabolic activation of fragrance substances is clearly needed to increase the safety for the consumer. Compounds suspected to act as prehaptens and/or prohaptens should be considered as allergens, unless it could be demonstrated that they do not become activated by one of the described pathways.

6. Retrieval of evidence and classification of fragrance substances

For a systematic review, a structured approach of identifying, grading and aggregating available information should be used. Regarding the classification of substances as allergens, a number of approaches have been suggested (158-160). The categorisation of skin sensitisers according to sensitising potency has also been proposed (161, 162). For this opinion, these discussions were extended to reconcile different perspectives and to arrive at a strategy that is both consistent and applicable in practice.

By default, positive human evidence (clinical data) overrides negative results obtained in animals. This implies that the observation of a sufficient number of positive clinical cases is more important than potency information derived from animal experiments (LLNA).

Cosmetovigilance information based on consumer complaints only is of limited value in the evaluation of sensitisation risk associated with cosmetic allergens, including fragrances, as it does not identify specific causative substances, and likely to severely under-estimate the frequency of contact dermatitis. An exception is the combination with qualified diagnostic work-up, as in the French REVIDAL/GERDA system (299); however, such data are generally published, thus publicly available, and considered in the present opinion.

6.1. Retrieval of evidence

A systematic search strategy was employed for the retrieval of clinical data, as outlined below. Experimental data are often not published hence the exact definition of the scope considered for the review is necessary and is given below. Additional LLNA data were reviewed, if identified by the search strategy, e.g. in chapter 8.1.2 and, as “additional information”, in Annex I of this opinion. This supplemental evidence was, however, not considered for the final categorisation in Table 13-2.

6.1.1. Search strategy for clinical data

Method of literature search:

1. Manual search of the issues of the journal “Contact Dermatitis” (for the 26 “annex substances”, which were re-evaluated in the present opinion, starting 1999) up to October 2010, identifying all studies with fragrance substances.
2. PubMed search of CAS numbers identified in the previous opinion, reviews and already identified clincial studies, respectively, and manual screening of identified publications (narrowed for the last 10 years for the 26 “annex substances”), if necessary narrowing the search results by adding “dermatitis” or “allergy”. For example, for citral: 5392-40-5 AND (dermatitis or allergy),
   translated into
   "5392-40-5"[EC/RN Number] AND
   (
   ("dermatitis"[MeSH Terms] OR "dermatitis"[All Fields])
   OR
   ("hypersensitivity"[MeSH Terms] OR "hypersensitivity"[All Fields] OR "allergy"[All Fields] OR "allergy and immunology"[MeSH Terms] OR ("allergy"[All Fields] AND "immunology"[All Fields]) OR "allergy and immunology"[All Fields])
)
3. Manual search of all RIFM reviews published in supplement issues of “Food and Chemical Toxicology[2]” in the past 20 years. In case of the least evidence on human sensitisation the substances were preliminarily selected and further research initiated.

[2] Food and Chemical Toxicology, Elsevier Ltd. http://www.sciencedirect.com/science/journal/02786915.

4. Consideration of the most important ("top 100") fragrance compounds in terms of volumes used (disregarding functional additives such as solvents) as supplied by the International Fragrance Association IFRA (personal communication 2010).
5. Consideration of fragrance compounds ranking 101 to 200 on the list of use volumes, if they were self-classified by manufacturers as skin sensitisers (R 43).

For the present systematic overview of available clinical data, only original studies were considered, as only these provide direct evidence, while other reviews, partly being based on the same original reports, only served to identify additional literature. In contrast, selected reviews, guidelines and similar publications were used as basis for methodological approaches (e.g., in section 11).

6.1.2. Collection of experimental (LLNA) data

The SCCS requested the International Fragrance Association (IFRA) to submit data on animal tests performed with fragrance substances, to be presented in a structured format. In response, industry submitted first a poster (163) and later a report consisting of LLNA protocol summaries on the 59 fragrance substances in the poster (164). No guinea pig studies were submitted. The SCCS has reviewed and analysed the report and the publications quoted in the report. A summary is given in chapter 8 and full data are given in Annex II. EC3 values on some additional fragrance substances in two published reviews (165, 166) have also been considered. Additional EC3 values may be available in the scientific literature and there may also be other unpublished data.

6.2. Grading of evidence

Assembled evidence has to be graded in two steps: (i) the quality of each single study, and (ii) the strength of evidence underlying the eventual classification as an allergen. Generally, studies (published or not) which are eligible for consideration will contribute to the final overall judgement to different degrees.

- Positive human data, if sufficiently demonstrated (point (i) below), will always over rule experimental (animal), *in vitro* or *in silico* data of similar internal validity, as they provide direct evidence on allergenicity in humans.
- Small study groups will contribute less precise information than larger studies of otherwise similar quality. As a minimum requirement, the size of the study groups and the numbers of events must be stated in the reports.

The following subsections will address special aspects of clinical and experimental studies, respectively.

6.2.1. Quality of a clinical study

Two major types of clinical studies must be distinguished because they provide a different scope of information:

(i) Case reports or small case series, focusing on patients with positive (test) reactions to the target substance, sometimes including a set of non-exposed, possibly non-diseased "control patients"; these should present a concise summary of all relevant aspects of the patient's history, diagnostic procedures and possibly further outcomes.

(ii) Clinical series in which results of a group of patients patch tested with the target substance, often combined with other substances, are presented. In the latter type of report, usually only a minority of patients tested show a positive reaction to the test substance. This implies that the majority of patients can be used to illustrate the proportion of irritant, doubtful and negative reactions. The degree of detail on the patients' histories is usually limited in such studies, compared to case reports.

Some of the basic quality criteria in clinical patch testing which should be considered are:

- Adherence to international patch test guidelines (32, 96).
- Material(s) tested should be characterised.
- Total number of patients tested must be given.
- Patient selection should be described.
- Relevance may be demonstrated either on a case-by-case basis, following pertinent guidelines, or in terms of a significant epidemiological association between sensitisation and exposure or valid markers of exposure.

Concerning relevance, it must be noted that while clinical relevance can provide important information (see 4.4.1), it is ideally based on comprehensive knowledge of prior exposures. Since the implementation of labelling 26 fragrances, previous exposure to these can often be ascertained in the assessment of relevance of a positive patch test reaction (44). However, exposure to substances not listed on a product ingredient label is obscure, except in very rare cases where elaborate diagnostics and chemical analyses are feasible (e.g. (167)). Thus, a lack of information on relevance (reported in studies) does not invalidate the impact of diagnosed contact sensitisation.

6.2.2. Quality of an experimental study

International guidelines such as the pertinent OECD guidelines for testing sensitisation have been developed and adopted. Experimental studies following these guidelines are considered as valid. However, a vast number of non-guideline studies are available and should be assessed on a case-by-case basis.

6.2.3. Quality of "other" evidence

Supporting evidence besides human and animal (experimental) data comprises *in vitro* test systems, *in chemico* experiments and structure activity relationships (SARs).

SAR analysis has at present no formal regulatory validation for skin sensitisation, nevertheless it may provide useful indicative information on sensitising potential when no or limited clinical or animal data are available.

SAR studies must consider a possible formation of haptens (allergens) from compounds able to act as prehaptens by, e.g. autoxidation outside the body as well as metabolic activation in the skin of compounds able to act as prohaptens (122, 168).

6.3. Aggregating evidence for a final conclusion

The criteria listed below are followed as a flow chart to arrive at a conclusion. This implies that if classification into one category is achieved, subsequent categories need not be considered. Based on the above criteria, fragrance substances were selected to be included in the present opinion if classified in one of the categories defined below.

6.3.1. Established contact allergen in humans

To qualify as an *established contact allergen*, the SCCS considers that *at least one* of the following two criteria must be met:

- At least two clinical series fulfilling the quality criteria from two different centres with cases of sensitisation, or at least three separate clinical series from different centres if a study, or studies, do not meet all quality criteria. (→ *sufficient human evidence present*)
  or
- Case reports from at least two independent centres describing more than two

patients altogether in whom clinically relevant contact sensitisation had unequivocally been proven (→ *sufficient human evidence present*)
or

- At least one clinical series fulfilling the quality criteria, together with at least one case report of clinically relevant contact sensitisation (→ *sufficient human evidence present*);
  or
- Experimentally induced sensitisation (e.g. unequivocally positive human maximisation tests/repeated insult patch test)[3] (→ *sufficient human evidence present*).

6.3.2. Established contact allergen in animals

To qualify as an *established contact allergen*, the following criterion must be met:

- At least one positive animal study carried out according to accepted guidelines, providing evidence of a sensitisation potential (→ sufficient animal evidence present).

6.3.3. Likely contact allergen, if human, animal and other evidence is considered

To qualify as an *likely contact allergen*, if classification as "established …" is not applicable, *at least two* of the following criteria must be met:

- Individual cases of allergic patch test reactions not fulfilling the requirements for sufficient evidence (→ *limited human evidence present*)
  or
- At least one positive non-guideline animal study, which should be evaluated on a case-by-case basis (→ *limited animal evidence present*)
  or
- Other evidence, e.g. results from *in chemico* experiments or *in vitro* tests or from structure-activity considerations based on sufficiently valid results for closely related compounds (→ *other evidence present*).

6.3.4. Possible contact allergen, if human, animal and other evidence is considered

To qualify as a *posible contact allergen*, if classification as "established …" or as "likely …" contact allergen is not applicable, *at least one* of the following criteria must be met:

- Individual cases of allergic patch test reactions not fulfilling the requirements for sufficient evidence (→ *limited human evidence present*)
  or
- At least one positive non-guideline animal study, which should be evaluated on a case-by-case basis (→ *limited animal evidence present*)
  or
- Other evidence, e.g. results from *in chemico* experiments or *in vitro* tests or from structure-activity considerations based on sufficiently valid results for closely related compounds (→ *other evidence present*).

[3] It should be noted that the SCCS considers such tests unethical (169. SCCP. Opinion concerning the predictive testing of potentially cutaneous sensiting cosmetic ingredients or mixtures of ingredients adopted by the SCCNFP during the 11th plenary session of 17 February 2000. 2000:).

6.4. Conclusions

The present opinion includes (i) a well-defined search strategy for retrieving pertinent evidence; (ii) a definition of criteria used to evaluate available evidence; and, finally (iii) a set of rules to categorise the substances with regard to the relevant toxicological endpoint, i.e. sensitisation in man, based on the evidence.

7. Reported fragrance allergens from the clinical perspective

In this chapter, clinical evidence regarding sensitisation to individual fragrance chemicals and to natural extracts (essential oils) is tabulated. In this report “single chemicals” refers to chemicals of natural or synthetic origin whose chemical identity is fully known. The term “natural extracts” refers to plant or animal derived mixtures of natural chemicals, for example lavender oil, whose composition may be variable and may or may not have been fully or partly established. Full information, including possible synonyms, structural formulas (in the case of single chemicals only), a short summary of available evidence and further information, e.g. on regulatory status, is presented in Annex I.

7.1. Tabular summary of evaluated individual fragrance chemicals

Regarding nomenclature, INCI names are used wherever possible. If an INCI name is not available, the perfuming name as listed by CosIng is used. Detailed information on the publications identified and considered for this report can be found in Annex I. Several substances are currently banned from the use in cosmetic products by Annex II of the Cosmetics Directive, based on concerns regarding one or more toxicological endpoints. While available clinical evidence regarding this set of substances is listed in Annex I, these substances have not been further evaluated and are thus not included in this chapter.

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

1. Established contact allergens in humans (→ *sufficient human evidence present*).
2. Substances with positive human data, which are, however, not sufficient to categorise as “established contact allergen in humans” (→ *limited human evidence present*).
3. Substances with negative human data, i.e. patch tests of patients with suspected contact allergy to fragrance ingredients which yielded negative results.
4. Substances eligible for inclusion (see beginning of chapter 6) for which no human data are available.

A critical point in understanding this scheme is that there is publication bias in reporting allergens. This is due to the fact that once a substance has been reported and accepted as a contact allergen in humans, further reports are less likely to be published unless they are part of a epidemiological survey or when there is a novel source of exposure. Moreover, the number of patients displaying positive test reactions obviously not only depends on the underlying prevalence of sensitisation, but also on how often a substance is patch tested. This implies that inclusion of an allergen or allergen mixture in the baseline patch test series (as for Fragrance Mix I and II, *Myroxylon pereirae* and HICC, and partly also other substances/mixtures) will yield the maximum possible number of cases. In contrast, patch testing in “special” series, e.g. as a break-down of single constituents of the respective mix in case of a positive reaction to the latter, or with application only in the case of strongly suspected fragrance intolerance, will mostly result in higher relative numbers than testing the same compound consecutively, but also in lower absolute numbers.

In Table 7-1, the single substances are listed with a semi-quantification of their impact which were categorised as established contact allergens in humans according to the criteria given in chapter 6.3.

Established contact allergens in humans, according to the criteria outlined in chapter 6.3.1, were categorised according to the number of patients reacting positively and to the number of patients tested, based on the publications considered (see annex I for references). The following categories were used:

+	Up to 10 positive test reactions reported
++	11 to 100
+++	101 to 1000
++++	> 1000

If a test allergen has been tested in less than 1,000 patients, “r.t.” (rarely tested) is added in the following tables. For this categorisation, absolute numbers of cases of sensitisation, and not the relative frequency of positive patch tests, were used, because relative frequencies depend heavily on the selection of patients for patch testing. Thereby, an important allergen tested routinely, in the baseline series, may yield 1 to 2% positive reactions (usually in several thousand patients), while an allergen tested in a selective fashion (in much fewer patients) may yield an even higher relative frequency. Moreover, case reports/series cannot be interpreted in terms of relative frequencies. The calculation of absolute numbers was based on all available literature, as detailed in the annex I to this opinion, i.e., regarding the 26 substances already listed in Annex III to the Cosmetics Directive includes data already evaluated in the previous opinion.

Table 7-1: Established contact allergens in humans (summary of evaluation as detailed in chapter 6.3). More detailed information forming the basis of this evaluation can be found in Annex I of this opinion.

INCI name (or, if none exists, perfuming name according to CosIng)	**CAS number**	**Comment: see text**
ACETYLCEDRENE	32388-55-9	+
AMYL CINNAMAL	122-40-7	++
AMYL CINNAMYL ALCOHOL	101-85-9	++
AMYL SALICYLATE	2050-08-0	+
trans-ANETHOLE	4180-23-8	+ (r.t.)
ANISYL ALCOHOL	105-13-5	+
BENZALDEHYDE	100-52-7	+
BENZYL ALCOHOL	100-51-6	++
BENZYL BENZOATE	120-51-4	++
BENZYL CINNAMATE	103-41-3	++
BENZYL SALICYLATE	118-58-1	++
BUTYLPHENYL METHYLPROPIONAL (Lilial®)	80-54-6	++
CAMPHOR	76-22-2 / 464-49-3	+ (r.t.)
beta-CARYOPHYLLENE (ox.)	87-44-5	Non-ox.: +, ox.: +
CARVONE	99-49-0 / 6485-40-1 / 2244-16-8	+ (r.t.)
CINNAMAL	104-55-2	+++
CINNAMYL ALCOHOL	104-54-1	+++
CITRAL	5392-40-5	+++
CITRONELLOL	106-22-9 / 1117-61-9 / 7540-51-4	++
COUMARIN	91-64-5	+++
(DAMASCENONE)	23696-85-7	+ (r.t.)

INCI name (or, if none exists, perfuming name according to CosIng)	**CAS number**	**Comment: see text**
ROSE KETONE-4		
alpha-DAMASCONE (TMCHB)[#]	43052-87-5 / 23726-94-5	++
cis-beta-DAMASCONE [#]	23726-92-3	+
delta-DAMASCONE [#]	57378-68-4	+
DIMETHYLBENZYL CARBINYL ACETATE (DMBCA)	151-05-3	+
EUGENOL	97-53-0	+++
FARNESOL	4602-84-0	+++
GERANIOL	106-24-1	+++
HEXADECANOLACTONE	109-29-5	+ (r.t.)
HEXAMETHYLINDANOPYRAN	1222-05-5	++
HEXYL CINNAMAL	101-86-0	++
HYDROXYISOHEXYL 3-CYCLOHEXENE CARBOXALDEHYDE *(HICC)*	31906-04-4 / 51414-25-6	++++
HYDROXYCITRONELLAL	107-75-5	+++
ISOEUGENOL	97-54-1	+++
alpha-ISOMETHYL IONONE	127-51-5	++
(DL)-LIMONENE	138-86-3	++ (non-ox.); +++ (ox.)
LINALOOL	78-70-6	++ (non-ox.) +++ (ox.)
LINALYL ACETATE	115-95-7	+
MENTHOL	1490-04-6 / 89-78-1 / 2216-51-5	++
6-METHYL COUMARIN[#]	92-48-8	++ (photo-allergy)
METHYL 2-OCTYNOATE	111-12-6	++
METHYL SALICYLATE	119-36-8	+
3-METHYL-5-(2,2,3-TRIMETHYL-3-CYCLOPENTENYL)PENT-4-EN-2-OL	67801-20-1	++ (r.t.)
alpha-PINENE and beta-PINENE	80-56-8 and 127-91-3, resp.	++
PROPYLIDENE PHTHALIDE	17369-59-4	+ (r.t.)
SALICYLALDEHYDE	90-02-8	++

[#] 76/768/EEC Annex III, part 1

[#] 76/768/EEC Annex III, part 1

INCI name (or, if none exists, perfuming name according to CosIng)	**CAS number**	**Comment: see text**
alpha-SANTALOL and beta-SANTALOL	115-71-9 and 77-42-9, resp.	++
SCLAREOL	515-03-7	+
TERPINEOL (mixture of isomers)	8000-41-7	+
alpha-TERPINEOL	10482-56-1 / 98-55-5	
Terpinolene	586-62-9	++
TETRAMETHYL ACETYLOCTAHYDRONAPHTHALENES	54464-57-2 / 54464-59-4 / 68155-66-8 / 68155-67-9	+
TRIMETHYL-BENZENEPROPANOL (Majantol)	103694-68-4	++
VANILLIN	121-33-5	++

Those substances which were categorised as +++ or more, i.e. those with the most reported cases, were also the top ranking substances in large series of patients tested with the 26 labelled fragrance ingredients ((44, 74) and additionally (170)). Geraniol is an exeption, as it was all negative in the Danish study (170), but was still among the top ten in the Dutch and German studies (44, 74), with prevalences of 0.5%-0.6% positives. Geraniol has, in addition, caused many cases of contact allergy in other areas of Europe (49).

The use of absolute numbers allows the pooling of studies with different selection criteria. Limonene and linalool were not tested in their oxidized forms in the three studies (44, 74, 170) and would not have been identified, if only these publications had been the basis of assessment.

It should be noted that oxidised fragrence terpenes with defined content of the major haptens formed after autoxidation have not been commercially available for testing in dermatology clinics. In the published clinical studies testing oxidised fragrence terpenes, the patch test preparations have been obtained specifically for the performed multicentre studies. From 2012, patch test preparations of oxidised limonene and oxidised linalool with defined content of the major allergens in the oxidation mixtures, i.e. the hydroperoxides, are commercially available (see also chapter 5).

Table 7-2 lists those substances which gave rise to a few reported cases of contact sensitisation only, or where results have been reported from just one clinical department. Thus, the level of evidence concerning human data must be regarded as *limited*, according to the definitions given in chapter 6.3.

Table 7-2: Fragrance substances with positive human data, which are, however, not sufficient to categorise as "established contact allergen in humans". More detailed information forming the basis of this evaluation can be found in Annex I of this opinion.

INCI name (or, if none exists, perfuming name according to CosIng)	**CAS number**	**Comment**	**Ref.**
AMBRETTOLIDE	7779-50-2	3.4% positive reactions in 178 patients	(171)
CARVACROL	499-75-2	2 of 28 patients	(Meynadier, after (172))
CUMINALDEHYDE	122-03-2	3 of 179 patients positive	(139)
CYCLOHEXYL ACETATE	622-45-7	0.5% positive of 218 selected patients	(173)
CYCLOPENTADECANONE	502-72-7	3 of 178 patients positive	(171)
trans-trans-delta-DAMASCONE	71048-82-3	1 positive HRIPT (2/15 with 1%)	(174)
2,3-DIHYDRO-2,2,6-TRIMETHYLBENZALDEHYDE	116-26-7	1 positive HRIPT (5 of 53)	(175).
DIMETHYLTETRAHYDRO BENZALDEHYDE	68737-61-1	2.3% positive reactions isomer mixture in 178 patients	(171)
ETHYLENE DODECANEDIOATE	54982-83-1	2 / 218 positive PT reactions	(173)
ETHYL VANILLIN	121-32-4	1 occupational case	(176)
HELIOTROPINE	120-57-0	6 / 1606 consecutive patients positive	(97)
HYDROXYCITRONELLOL	107-74-4	6.0% positive PT reactions in 218 patients	(173)
ISOAMYL SALICYLATE	87-20-7	1 positive in 179 patients, possibly "excited back syndrome" 0 / 95 in another study with <= 1/10 of above test conc.	(139) (70)
ISOLONGIFOLENEKETONE	33407-62-4	1 / 178 patients	(171)
METHOXYCITRONELLAL	3613-30-7	Positive PT data of unknown validity by Nakayama et al. in 22/137 patients.	(177)
METHOXYTRIMETHYLHEPTANOL	41890-92-0	0.9% positive PT	(173)
METHYL p-ANISATE	121-98-2	1 / 182 patients positive	(178)
METHYL CINNAMATE	103-26-4	6 / 142 patients positive	(179)
METHYL DIHYDROJASMONATE	24851-98-7	3 / 1606 patients positive 0 / 100	(97) (70)
METHYLIONANTHEME	55599-63-8	1 case	(180)
5-METHYL-alpha-IONONE	79-69-6	5 / 1606	(97)
METHYL OCTINE CARBONATE	111-80-8	1 case	(181)

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment	Ref.
MYRCENE	123-35-3	1 / 1511 positive to oxidized myrcene	(133)
MYRTENOL	515-00-4	2 HRIPTs with 1 pos. each	(182)
NEROL	106-25-2	6.0% positive	(173)
Nerolidol (isomer not specified)	7212-44-4	Few, unconfirmed pos. cases according to RIFM review	(183)
NOPYL ACETATE	128-51-8	2 / 179 positive, possibly “excited back syndrome”	(139)
PHENETHYL ALCOHOL	60-12-8	1 / 179; 0 / 100	(139) (70)
PHENYLACETALDEHYDE	122-78-1	1.1% of 182 positive. 1 case	(178) (184)
PHENYLPROPANOL	122-97-4	2 / 218	(173)
PHYTOL	150-86-7	1 case in human max. test	(185)
RHODINOL	6812-78-8	Several pos. HRIPTs, clinical data of uncertain validity	(186)
trans-ROSE KETONE-5	39872-57-6	2 / 22 pos. HRIPT	(187)

For a number of substances negative patch tests results were obtained, usually in rather small patient samples (max. 313 patients). For some of these substances exposure is substantial, according to data submitted from IFRA. It should be noted that a negative result does not rule out a notable sensitisation prevalence, as the study size has to be larger than, e.g. n=298 to yield a 95% CI which excludes a prevalence of 1% and larger than n=597 to exclude a prevalence of 0.5%.

Table 7-3: Fragrance substances with negative human data, i.e. patch tests of patients with suspected contact allergy to fragrance ingredients which yielded negative results.

INCI name (or, if none exists, perfuming name according to CosIng)	**CAS number**	**Results / Comment**	**Ref.**
6-ACETYL-1,1,2,4,4,7-HEXAMETHYLTETRALINE	21145-77-7	0 / 313 consecutive patients in 2 centres	(70)
AMYLCYCLOPENTANONE	4819-67-4	0 / 178	(171)
BENZYL ACETATE	140-11-4	0 / 100 consecutive patients in 1 centre observed	(70)
2-TERT-BUTYLCYCLOHEXYL ACETATE	88-41-5	0 / 313 consecutive patients in 2 centres	(70)
4-tert.-Butylcyclohexyl acetate	32210-23-4	0 / 107 consecutive patients in 1 centre observed	(70)
6-ETHYLIDENEOCTAHYDRO-5,8-METHANO-2H-BENZO-1-PYRAN	93939-86-7	0 / 178	(171)
3α,4,5,6,7,7α-HEXAHYDRO-4,7-METHANO-1H-INDEN-5(OR 6)-YL ACETATE	54830-99-8	0 / 313 consecutive patients in 2 centres	(70)
HEXYL SALICYLATE	6259-76-3	0 / 218 “top 100” substance and classified as R43	(173)
HIBISCOLIDE	6707-60-4	0 / 178	(171)
alpha-IONONE	127-41-3	0 / 205	(70)
beta-IONONE	79-77-6	0 / 205 “top 100” substance	(70)
ISOBORNYL ACETATE	125-12-2	0 / 107 “top 100” substance	(70)
METHYL ANTHRANILATE	134-20-3	0 / 91 “top 100” substance	(188)
METHYL IONONE (mixture of isomers)	1335-46-2	0 / 100 “top 100” substance	(70)
OXALIDE	1725-01-5	0 / 178	(171)
TERPINEOL ACETATE (Isomer mixture)	8007-35-0	0 / 106 “top 100” substance	(70)
alpha-TERPINYL ACETATE	80-26-2	0 / 179	(139)
TRIMETHYL-PROPYLCYCLOHEXANEPROPANOL	70788-30-6	0 / 178	(171)

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

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

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

[#] Annex III, part 1

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

7.2. Tabular summary of evaluated natural extracts/essential oils

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

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

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

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

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

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

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

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

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

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

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

[#] 76/768/EEC Annex III, part 1

[#] 76/768/EEC Annex III, part 1

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

Notes: r.t. Rarely tested.

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

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

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

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

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

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

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

7.3. Conclusions

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

8. Animal data

8.1. Predictive tests and sensitising potency categories

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

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

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

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

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

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

---

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

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

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

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

[10] Regulation No. 1272/2008.

8.1.1. LLNA data

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

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

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

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

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

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

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

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

M: EC3 based on molar concentration

8.1.2. LLNA data on oxidised fragrance substances

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

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

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

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

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

8.2. Methodological considerations

EC3 mean values

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

Vehicle

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

Number of doses and animals

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

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

Units for concentrations

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

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

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

8.3. Summary of animal data by LLNA

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

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

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

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

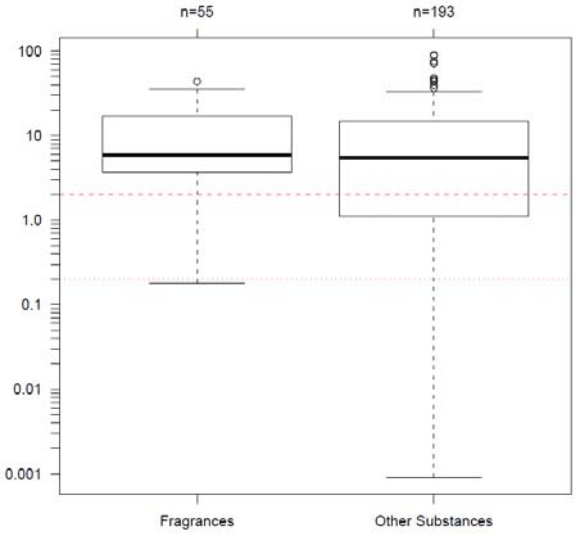


Figure 8-1: The distribution of fragrance chemicals and a variety of other chemicals (e.g. biocides, dyes, plastic materials), taken from the three references (164-166), are depicted as boxplots on a logarithmic scale. The bottom of the box denotes the 1st quartile (25% percentile), the thick line in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

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

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

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

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

8.4. Conclusions

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

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

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

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

The relationships between molecular structure and reactivity that form the basis for structural alerts are based on well established principles of mechanistic organic chemistry (214). Examples of structural alerts are aliphatic aldehydes (alerting to the possibility of sensitisation via a Schiff base reaction with protein amino groups), and α,β-unsaturated carbonyl groups, C=C-CO- (alerting to the possibility of sensitisation via Michael addition of protein thiol groups). Major mechanistic reactivity domains have been discussed in detail by Aptula and Roberts (215). Prediction of the sensitisation potential of compounds that can act via abiotic or metabolic activation (pre- or prohaptens) is more complex compared to that of compounds that act as direct haptens without any activation. The autoxidation patterns can differ due to differences in the stability of the intermediates formed, e.g. it has been shown that autoxidation of the structural isomers linalool and geraniol results in different major haptens/allergens. Moreover, the complexity of the prediction increases further for those compounds that can act both as pre- and prohaptens. In such cases, the impact on the sensitisation potency depends on the degree of abiotic activation (e.g. autoxidation) in relation to the metabolic activation.

These structural alerts can be applied by computerized expert systems, i.e. *in silico* or by estimations made by organic chemists (*in cerebro)* using their experience. When an organic chemist looks at a chemical structure, they recognise parts of the structure that they can associate with reactivity, the type of reactivity (i.e. assign the reaction mechanistic domain), and other features of the molecular structure that will affect the reactivity positively or negatively. Human experts should be aware of the complexities, and how structural modification can alter the reactivity associated with structural alerts, etc. Importantly, they can also recognise where there are unfamiliar structural features whose effects they cannot confidently predict. In such cases they can call for experimental chemistry work (*in chemico*) to be done to ascertain the presence or nature of, and degree of reactivity. *In chemico* methods include organic chemistry experimentation to identify chemical reaction products from oxidation and/or reaction with model nucleophiles, identification of mechanisms of reaction. In so called *in chemico* reactivity methods, the ability of a specific chemical to react with selected peptides is determined so as to predict the sensitisation potential of the chemical under investigation (216, 217). To make *in chemico* reactivity methods able to predict the activity of prohaptens, the addition of horseradish peroxidase and hydrogen peroxide oxidation system has been tested to model the enzymatic oxidation in the skin (218, 219).

Although computerized expert systems are derived from input by human experts, they are less well able to capture the subtleties of structure reactivity relationships, and they sometimes fail to detect aspects of chemistry that are obvious to organic chemists. Human experts should be aware of the complexities, as well as how structural modification can alter the reactivity associated with structural alerts, etc.

The SAR evaluation made in the section below is based on *in cerebro* alerts applied by organic chemists.

Depending on the type of reactivity (the **reaction mechanistic domain**), it is sometimes possible to make a quantitative prediction of potency in the LLNA, which can be used to predict potency in humans relative to related known human sensitisers. These predictions use quantitiative mechanistic models (**QMMs**) based on reactivity expressed quantitatively

by model parameters, and sometimes in combination with hydrophobicity. For example, potency of aliphatic aldehydes and ketones (the Schiff base domain) in the LLNA is modelled by a combination of reactivity and hydrophobicity (220), whereas the LLNA potency of DNCB analogues (the S_NAr domain) is well modelled by reactivity alone (221).

QMMs aiming not only to predict the potential to be a sensitiser but also to predict the potency, promise to be a useful tool in non-animal based risk assessment for skin sensitisation. However, in the field of fragrance substances there are major gaps in our present ability to apply QSAR/QMM. This is largely because many of the fragrance substances of interest have the potential to act via abiotic or metabolic activation (pre- and/or prohaptens, i.e. they themselves are only weak or non-sensitisers, but have the potential to be activated to form more potent sensitisers. Resulting sensitisation potency will depend on the extent of activation and the nature of the resulting products. It is possible to apply SAR analysis to identify these plausible possibilities, but QSAR modelling for these cases is not yet developed. However, much progress has been made in identifying structural alerts for the various activation mechanisms that have been recognised. This is reviewed by Karlberg et al. (122).

Chemicals with no structural alerts for direct reactivity, or for known activation mechanisms, and no unfamiliar structural features that might be associated with as yet unidentified activation mechanisms, can be predicted to be non-sensitising. Chemicals that do have alerts for reactivity (direct or via activation) are not necessarily sensitisers – they may be insufficiently reactive and/or insufficiently hydrophobic.

Substances meeting the inclusion criteria (see chapter 6), for which, however, no categorisation as established contact allergen in humans or established contact allergen in animals was possible, have been assessed for structural alerts. The results are presented in four tables based on the prediction made for the actual substance. The following SAR assessments have been used:

- Predicted sensitiser; structural alerts:
  Compounds containing structural alerts comprising direct reactive compounds and for compounds that after specific abiotic or metabolic activation (prohaptens and prehaptens) can be predicted to be sensitisers by structural comparison to known allergens.
- Possible sensitiser; structural alerts:
  Compounds containing structural alerts that by comparison to known allergens with similar structures were expected to be less reactive and hence less likely to be sensitising. Also compounds with structural alerts indicating a possible abiotic or metabolic activation (possible prehaptens or prohaptens) but with no structural data available for comparison, were included in this group. Consequently, a possible sensitiser may turn out to be a non sensitiser when tested in vivo.
- Predicted non-sensitiser (NS); no obvious structural alerts
- Not predictable due to insufficient/conflicting data

Table 9-1: Predicted sensitisers.

Substance (INCI) name	CAS number	Structural alerts
p-tert.-Butyldihydrocinnamaldehyde[§]	18127-01-0	Schiff base
Citronellal	106-23-0	Schiff base and possible prehapten
Citronellyl nitrile	51566-62-2	Possible prehapten
Decanal	112-31-2	Schiff base
3,7-Dimethyl-1,6-nonadien-3-ol	10339-55-6	Prehapten
Geranyl acetate	105-87-3	Prehapten and prohapten

Isoamyl salicylate	87-20-7	Acyltranfer agent
Methyl cinnamate	103-26-4	Michael acceptor
Methylundecanal	110-41-8	Schiff base
Myrcene	123-35-3	Prehapten
Nerol	106-25-2	Prehapten and prohapten
Nerolidol (isomer not specified)	7212-44-4	Possible prehapten
Oxacyclohexadecenone	34902-57-3	Michael acceptor
Phenethyl salicylate	87-22-9	Acyltransfer agent
trans-Rose ketone-5	39872-57-6	Michael acceptor and possible prehapten

Note: § Classified as R43.

Table 9-2: Possible sensitisers.

Substance (INCI) name	**CAS number**	**Structural alerts**
Ambrettolide	7779-50-2	Possible prehapten
Amylcyclopentanone	4819-67-4	Schiff base; the combination of reactivity and hydrophobicity may be enough to confer sensitisation
Benzyl acetate	140-11-4	Prohapten via hydrolysis leading to benzyl alcohol
Carvacrol	499-75-2	Possible prehapten
Cuminaldehyde	122-03-2	Schiff base and possible prehapten
alpha-Cyclohexylidene benzeneacetonitrile	10461-98-0	Possible Michael acceptor
Cyclopentadecanone	502-72-7	Schiff base; the combination of reactivity and hydrophobicity may be enough to confer sensitisation
trans-beta-Damascone	23726-91-2	Possible Michael acceptor
trans-trans-delta-Damascone	71048-82-3	Possible Michael acceptor and possible prehapten
gamma-Damascone	35087-49-1	Possible Michael acceptor and possible prehapten
Dihydromyrcenol	18479-58-8	Possible prehapten
2,3-Dihydro-2,2,6-trimethylbenzaldehyde	116-26-7	Possible Michael acceptor and possible prehapten and possible prohapten
2,4-Dimethyl-3-cyclohexen-1-carboxaldehyde §	68039-49-6	Schiff base and possible prehapten
Dimethyltetrahydro benzaldehyde	68737-61-1	Schiff base and possible prehapten
6-Ethylideneoctahydro-5,8-methano-2H-benzo-1-pyran	93939-86-7	Possible prehapten
2-Ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)-2-buten-1-ol	19-61-6	Possible prehapten
Ethyl vanillin	121-32-4	Complex
Heliotropine	120-57-0	Possible prohapten
3α,4,5,6,7,7α-Hexahydro-4,7-methano-1H-inden-5(or 6)-yl	54830-99-8	Possible prehapten

Substance (INCI) name	CAS number	Structural alerts
acetate		
Hexahydro-methanoindenyl propionate	68912-13-0	Possible prehapten
Ionone isomeric mixture	8013-90-9	Possible Michael acceptor and possible prehapten
alpha-Ionone	127-41-3	Possible Michael acceptor and possible prehapten
beta-Ionone	79-77-6	Possible Michael acceptor
Isobergamate	68683-20-5	Possible prehapten
Isolongifoleneketone	33407-62-4	Schiff base; the combination of reactivity and hydrophobicity may be enough to confer sensitisation
Longifolene[§]	475-20-7	Possible prehapten
Methoxycitronellal	3613-30-7	Schiff base
Methyl decenol	81782-77-6	Possible prehapten
Methyl ionone (mixture of isomers)	1335-46-2	Possible Michael acceptor and possible prehapten
Methylionantheme	55599-63-8	Possible Michael acceptor and possible prehapten
5-Methyl-alpha-ionone	79-69-6	Possible Michael acceptor and possible prehapten
Myrtenol	515-00-4	Possible prehapten
Nopyl acetate	128-51-8	Possible prehapten
Phytol	150-86-7	Possible prehapten and/or prohapten
Rhodinol	6812-78-8	Possible prehapten
Terpineol acetate (isomer mixture)	8007-35-0	Possible prehapten
alpha-Terpinyl acetate	80-26-2	Possible prehapten
Tricyclodecenyl propionate	17511-60-3	Possible prehapten
Verdyl acetate	2500-83-6/ 5413-60-5	Possible prehapten

Note: [§] Classified as R43.

Table 9-3: Predicted non-sensitisers with no obvious structural alerts.

Substance (INCI) name	CAS number	Structural alerts
6-Acetyl-1,1,2,4,4,7-hexamethyltetraline	21145-77-7	
Benzyl acetone	2550-26-7	Schiff base; the combination of reactivity and hydrophobicity may **not** be enough to confer sensitisation
2-tert.-Butylcyclohexyl acetate	88-41-5	
4-tert.-Butylcyclohexyl acetate	32210-23-4	
Cyclohexyl acetate	622-45-7	
Diphenyl ether	101-84-8	

Substance (INCI) name	CAS number	Structural alerts
Ethyl 2-methylbutyrate	7452-79-1	
Ethylene dodecanioate	54982-83-1	
Ethylene brassylate	105-95-3	
Eucalyptol	470-82-6	
Hexyl acetate	142-92-7	
Hibiscolide	6707-60-4	
Hydroxycitronellol	107-74-4	However, dehydration followed by autoxidation could give sensitising impurities
Isoamyl acetate	123-92-2	
Isobornyl acetate	125-12-2	
Methoxytrimethylheptanol	41890-92-0	
Methyl p-anisate	121-98-2	
Methyl anthranilate	134-20-3	
Methylbenzyl acetate	93-92-5	
Methyl dihydrojasmonate	24851-98-7	Schiff base; the combination of reactivity and hydrophobicity may **not** be enough to confer sensitisation
Oxalide	1725-01-5	
Pentadecalactone	106-02-5	
Phenethyl acetate	103-45-7	
Phenethyl alcohol	60-12-8	
Phenoxyethyl isobutyrate	103-60-6	
Phenylisohexanol	55066-48-3	
Phenylpropanol	122-97-4	
Tetrahydrolinalool	78-69-3	
Tetrahydro-methyl-methylpropyl)-pyran-4-ol	63500-71-0	
Trimethylhexyl acetate	58430-94-7	
Trimethyl-propylcyclohexanepropanol (tmch)	70788-30-6	
gamma-Undecalactone	104-67-6	

Table 9-4: Not predictable.

Substance (INCI) name	CAS number	Structural alerts
Anisaldehyde	123-11-5	Due to insufficient /conflicting data; structural similarities to benzaldehyde suggest certain activity in man
Trichloromethyl phenyl carbinyl acetate	90-17-5	Due to insufficient /conflicting data
Methyl beta-naphthyl ether	93-04-9	Due to insufficient /conflicting data

9.1. General results

From this work with the included SAR predictions, the following observations can be made.

- For substances for which sufficient experimental/clinical evidence is missing, SAR analyses have been performed to predict a probable or possible risk of allergenic (sensitising) effect. These predictions are based on chemical reactivity and the recognition of structural features in a substance that are in common with the structural features that have been shown to cause sensitisation from other substances. In cases where the SAR analysis indicates a sensitisation potential, the substance should be investigated further to confirm or reject the conclusion drawn from the SAR analysis.
- Prediction of the sensitisation potential of compounds that can act via abiotic or metabolic activation (pre- or prohaptens) becomes more complex compared to that of compounds that act as direct haptens without any activation.
- The complexity of the prediction increases further for those compounds that can act both as prehaptens and prohaptens.
- Prediction of the sensitisation potential of compounds that can act as prehaptens is further complicated by the fact that the autoxidation patterns can differ due to differences in the stability of the intermediates formed, e.g. it has been shown that autoxidation of the structural isomers of linalool and geraniol results in different major haptens/allergens.

9.2. Conclusions

The SAR evaluation made in this section is based on *in cerebro* alerts applied by organic chemists.

- Applying only mechanism-based QSAR (QMM) as a tool in non-animal based risk assessment for skin sensitisation is of limited value for fragrance substances. This is due to major information gaps in the present model when addressing substances that act via abiotic or metabolic activation, and the high incidence of such substances in fragrances.
- Quantitative structure activity relationship (QSAR) models should be further developed, combining, as appropriate, information from *in silico*, *in chemico* and *in vitro* methods.
- SAR, as performed here, is only one consideration in the overall weight of evidence.

10. Exposure

Exposure to fragrance chemicals and other potential allergens is most commonly by direct skin contact. Exposures to fragrance chemicals occur from:

- Personal cosmetic use;
- Detergents and other household products;
- Medicaments;
- Occupation, i.e. personal hygiene, manufacturing ingredient(s), product in work process, plant materials;
- Secondary exposure from another individual (e.g. spouse, child);
- Toys;
- Oral intake;
- Airborne exposure.

Factors that are important for both the induction and elicitation of contact allergy are:

- Dose per unit area;
- Vehicle effects including penetration enhancers;
- Presence of skin irritants;
- Presence of other allergens (combination effects);
- Duration of skin exposure;
- Frequency of applications;
- Anatomical sites of exposure;
- Condition of the skin (barrier function impairment, pre-existing inflammation);
- Occlusion (e.g. in flexures, under clothing and personal protective equipment).

Fragrance mix ingredients are commonly present in cosmetic formulations (71, 222-224). Cosmetics based on natural ingredients may contain fragrance allergens at a higher concentration than other cosmetic products (225). The clinical significance of exposure to natural extracts is difficult to determine as there is often “hidden and variable” exposure to important and potent allergens in natural products.

10.1. Concentrations and quantities used

Consumers are exposed in daily life to fragrance chemicals from a large variety of products, such as cosmetics, toys, detergents and other cleaning products, etc. The fragrance exposure may be via dermal and/or inhalation route. With respect to “Terms of Reference” to the SCCS, only dermal exposure from cosmetics is addressed in this opinion. As cosmetics are the perfumed products most commonly used in daily life, potential fragrance allergens identified by the use of cosmetics also represent the exposures of these chemicals from other product categories. In recent years, it has become a trend to add fragrance chemicals to many other types of consumer products, such as children’s toys, toilet paper and nappies, which may contribute significantly to the fragrance exposure of the consumer by the dermal route.

Factors for the fragrance exposure assessment by the dermal route require knowledge on:

- Product types (categorisation of scented products) used by the consumer.
- Market survey (impression of the qualitative and quantitative contents of different allergens in consumer products).

- Hydrolysis, metabolism or oxidation of a fragrance material, which may generate a potential skin allergen.
- Chemicals in the product matrix, which may significantly enhance or reduce dermal absorption of a fragrance material.

Fragrance materials, both defined chemical substances and natural mixtures of chemicals (essential oils), are used in all types of cosmetic products: perfumes, eau de cologne, eau de perfume (EDP), and eau de toilette (EDT), aftershave lotion, deodorants, skin care products, skin cleansers, make-up cosmetics, hair care products, and oral care products, etc. However, some unscented cosmetic products have also reached the market in the last decade. Products containing the highest concentration of fragrance chemicals are perfumes, followed by eau de cologne, eau de perfume (EDP) and eau de toilette (EDT). Concentrations of fragrance chemicals in deodorant products are lower than those in EDT/EDP products, but still significant. Aftershave products also contain relatively high amounts of fragrance chemicals. Other cosmetic products contain relatively low amounts, 0.1-1% of fragrance compound, compared to up to 30% fragrance compound in EDT/EDP (226). The fragrance compound are mixtures of 20 to over 200 synthetic fragrance chemicals or natural fragrance materials (essential oils), selected from over 3,000 fragrance materials (226). For the exposure assessment, levels of fragrance chemicals in cosmetics containing significant amounts of fragrance materials (i.e. EDP/EDT/aftershave/deodorant) should be selected. It may not be possible to detect/measure the amounts of all fragrance chemicals when present in highly diluted form in a cosmetic product such as skin care products, make-up cosmetics etc. On the other hand, if a fragrance is evaluated safe for use when present in significant amounts in a product, it will also be safe for use in other products. Also the analysis of trend of the use of individual fragrance materials should be based on monitoring their contents in fine perfumes and deodorants.

Ninety of the 100 fragrance materials used in annual volumes > 175 tons in perfume formulations are fragrances and the remaining ten are used for other functions such as solvents or antioxidants (IFRA, personal communication 2010).

Among the 26 fragrances currently requiring individual labelling, amyl cinnamal, benzyl benzoate, benzyl salicylate, butyl phenyl methyl propional, citral, citronellol, coumarin, eugenol, geraniol, hexyl cinnamal, hydroxyisohexyl 3-cyclohexene carboxyaldehyde (HICC), alpha-isomethyl ionone, and linalool are used in volumes greater than 175 ton. α-Amylcinnamyl alcohol, anisyl alcohol, benzyl alcohol, benzyl cinnamate, cinnamal, cinnamyl alcohol, farnesol, hydroxycitronellal, isoeugenol, *d*-limonene, methyl-2-octynoate, oak moss (*Evernia prunastri),* tree moss (*Evernia furfuracea*) are used in volumes less than 175 ton.

According to the information from the fragrance industry, 80% of the total fragrance chemical volume is used in cosmetics and 20% in household products.

Since the implementation of the regulation of labelling of 26 fragrance substances in cosmetic products, qualitative information on fragrance exposure from cosmetics is provided in some market surveys performed on cosmetics (Table 10-1, (227)) and (Table 10-2, (228)) and on consumer products including cosmetics (Table 10-3, (229); Table 10-4, (115); and Figure 10-1, (105)). Thus, the implementation of the regulation of fragrance allergens in detergents (Directive 648/2004/EC), similar to that for cosmetics, has also added to the knowledge of fragrance exposure to the consumer. These market surveys revealed that fragrance ingredients which are potent allergens and frequently cause allergies in consumers are used as ingredients in consumer products including cosmetics. The results of these surveys further revealed that limonene and linalool were the most commonly used fragrance chemicals in cosmetics, while anisyl alcohol, cinnamal, α-amylcinnamyl alcohol, oak moss and tree moss were the least used fragrance ingredients in cosmetics and other consumer products. In general, the most potent allergens were also the most infrequently used ingredients. Prior to the regulation of the 26 allergens, analysis of 21 selected fragrance chemicals in deodorants also revealed additional 66 potential allergens in these products on the basis of structure activity relationship (230).

Table 10-1: Presence in children's cosmetics of the 26 fragrance substances that are required to be labelled in cosmetics (227).

Fragrance substance		% Products labelled to contain the fragrance substance
INCI name	**CAS number**	
Amyl cinnamal	122-40-7	8.2
alpha-Amylcinnamyl alcohol	101-85-9	2.9
Anise alcohol	105-13-5	0
Benzyl alcohol	100-51-6	9.6
Benzyl benzoate	120-51-4	9.1
Benzyl cinnamate	103-41-3	2.9
Benzyl salicylate	118-58-1	9.6
Butyl phenyl methyl propional	80-54-6	7.7
Cinnamal	104-55-2	1
Cinnamyl alcohol	104-54-1	6.7
Citral	5392-40-5	8.2
Citronellol	106-22-9	10.5
Coumarin	91-64-5	4.8
Eugenol	97-53-0	7.2
Farnesol	4602-84-0	2.9
Geraniol	106-24-1	12
Hexyl cinnamal	101-86-0	10.1
Hydroxycitronellal	107-75-5	6.3
Hydroxyisohexyl-3-cyclohexene carboxyaldehyde	31906-04-4	5.8
Isoeugenol	97-54-1	0.5
Alpha-isomethyl ionone	127-51-5	5.8
d-Limonene	5989-27-5	23.1
Linalool	78-70-6	21.6
Methyl-2-octynoate	111-12-6	0
Evernia prunastri/oak moss	90028-68-5	0
Evernia furfuracea/tree moss	90028-67-4	0

Table 10-2: Usage trends in deodorants of fragrance chemicals that are required to be labelled in cosmetics.

Fragrance substance		**88 products investigated in 2007 (228)**			**70 products investigated in 1998 (231)**	
INCI name	**CAS number**	**% Products labelled to contain the fragrance**	**Content in 23 selected products**		**Content in all 70 products**	
			% Products found to contain the fragrance	**Range(ppm)**	**% Products found to contain the fragrance**	**Range (ppm)**
Amyl cinnamal▪	122-40-7	10.2	17	2.3-165	31	1-617
alpha-amyl cinnamyl alcohol	101-85-9	-	-	-	n.a.	n.a.
Anise alcohol	105-13-5	2.3	9	1, 51	n.a.	n.a.
Benzyl alcohol	100-51-6	17.1	26	32-166	76	1-629*
Benzyl benzoate	120-51-4	25.0	48	3-4054	71	1-1075
Benzyl cinnamate	103-41-3	3.4	9	74, 143	n.a.	n.a.
Benzyl salicylate	118-58-1	39.8	48	136-5279	49	1-18758
Butyl phenyl methyl propional	80-54-6	48.9	70	1-5455	51	1-3732
Cinnamal▪	104-55-2	1.1	4	5	17	1-424
Cinnamyl alcohol▪	104-54-1	12.5	48	2-503	39	6-1169
Citral▫	5392-40-5	26.1	44	39-554	n.a.	n.a.
Citronellol▫	106-22-9	65.9	91	1-5848	81	1-5585
Coumarin▫	91-64-5	33.0	52	3.8-1255	57	1-1411
Eugenol▪	97-53-0	27.3	30	1-514	57	1-2355
Farnesol▫	4602-84-0	14.8	39	9-1791	n.a.	n.a.
Geraniol▪	106-24-1	48.9	87	1-399	76	1-1178

Fragrance substance		88 products investigated in 2007 (228)			70 products investigated in 1998 (231)	
Hexyl cinnamal▫	101-86-0	33.0	48	1-4434	71	2-1684
Hydroxycitronellal▪	107-75-5	27.3	70	1-1746	50	1-1023
HICC▫	31906-04-4	33.0	74	1-4431	53	1-1874
Isoeugenol▪	97-54-1	9.1	35	1-138	29	1-458
Alpha-isomethyl ionone	127-51-5	46.6	65	6-2588	61	1-2765
D-Limonene◦	5989-27-5	53.4	70	1022-11386	n.a.	n.a.
Linalool◦	78-70-6	53.4	96	8-3447	97	9-1927
Methyl-2-octynoat◦	111-12-6	1.1	-	-	n.a.	n.a.
Evernia prunastri▪/oak moss	90028-68-5	4.6	n.a.	n.a.	n.a.	n.a.
Evernia furfuracea▪/tree moss	90028-67-4	2.3	n.a.	n.a.	n.a.	n.a.

Notes: HICC Hydroxyisohexyl-3-cyclohexene carboxyaldehyde.
- Fragrance not detected in any product.
n.a. Not analysed.
* Benzyl alcohol could not be determined in 49% of the products due to interference.

The most common fragrance allergens are contained in the two mixtures, which are used for diagnosing fragrance allergy, called Fragrance Mix I (▪) and Fragrance Mix II (▫), besides the oxidation product of terpens (◦), and tree moss extract are common allergens. Methyl-2-octynoate is an extreme, but rare allergen.

Table 10-3: Frequency of occurrence in consumer products of the 26 fragrance allergens that are required to be labelled in cosmetics and detergents (229).

INCI name of fragrance	PCP (n = 70)	MP (n = 59)	HP (n = 57)	WP (n = 44)	Cos (n = 39)	Deo (n = 17)	Dent (n = 14)	Total (n = 300)
Linalool	46	47	17	42	26	12	0	190 (63%)
Limonene	34	45	29	43	18	11	9	189 (63%)
Citronellol	23	24	21	37	25	15	0	145 (48%)
Geraniol	19	26	15	36	18	12	0	126 (42%)
BPMP	30	27	21	27	13	8	0	126 (42%)
Hexyl cinnamal	37	20	22	22	14	10	0	125 (42%)
Benzyl salicylate	23	23	10	31	15	12	0	114 (38%)
Alpha-isomethyl ionone	15	20	7	24	28	10	0	104 (35%)
Coumarin	12	27	8	23	12	8	0	90 (30%)
Lyral™	17	24	3	24	15	5	0	88 (29%)
Eugenol	13	26	4	22	6	6	3	80 (27%)
Citral	2	28	6	29	7	2	0	74 (25%)
Benzyl benzoate	8	9	3	31	11	8	0	70 (23%)
Benzyl alcohol	9	8	1	30	9	3	1	61 (20%)
Hydroxycitronellal	5	6	1	30	6	4	0	52 (17%)
Isoeugenol	2	5	0	17	0	3	0	27 (9%)
Cinnamic alcohol	4	2	0	13	4	2	0	25 (8%)
Farnesol	1	3	0	17	2	0	0	23 (8%)
Amyl cinnamal	5	0	3	7	5	2	0	22 (7%)
Cinnamal	3	4	0	7	0	0	3	17 (6%)
Evernia prunastri/oak moss	0	3	0	5	5	0	0	13 (4%)
Benzyl cinnamate	2	0	0	8	0	0	0	10 (3%)
Evernia furfuracea/tree moss	1	5	0	3	0	0	0	9 (3%)
Anisyl alcohol	0	0	0	1	0	0	0	1 (0·3%)
Amyl cinnamic alcohol	0	0	0	0	0	0	0	0
Methyl heptine carbonate	0	0	0	0	0	0	0	0

INCI, International Nomenclature of Cosmetic Ingredients; PCP, personal care products; MP, men's products; HP, household products; WP, women's perfumes; Cos, cosmetics; Deo, deodorants; Dent, dental products; BPMP, butyl phenyl methyl propional; Lyral™, hydroxy-isohexyl-3-cyclohexene carboxaldehyde.

Table 10-4: Frequency in 516 consumer products of the 26 fragrance substances that are required to be labelled in cosmetics* (115).

Fragrance substance INCI name	**% Product containing the chemical**
D-Limonene	48.3
Linalool	35.8
Butyl phenyl methyl propional	24.8
Geraniol	22.1
Alpha-isomethyl ionone	21.7
Hexyl cinnamal	21.3
Citonellol	21.1
Benzyl salicylate	18.6
Coumarin	17.0
Eugenol	15.7
Benzyl alcohol	15.3
Benzyl benzoate	14.7
Hydroxyisohexyl-3-cyclohexene carboxyaldehyde	12.8

Fragrance substance INCI name	% Product containing the chemical
Citral	11.6
Hydroxycitronellal	10.8
Amyl Cinnamal	7.9
Anise alcohol	7.0
Cinnamyl alcohol	6.4
Farnesol	3.9
Isoeugenol	3.1
Cinnamal	2.5
Benzyl cinnamate	2.3
Amylcinnamyl alcohol	1.9
Methyl-2-octynoate	1.0
Evernia prunastri▪/oak moss	0.8
Evernia furfuracea▪/tree moss	0.4

Note: * Consumer Products: Cosmetics and household products with labelling of the 26 fragrance allergens. The content of these fragrances was confirmed by chemical analysis.

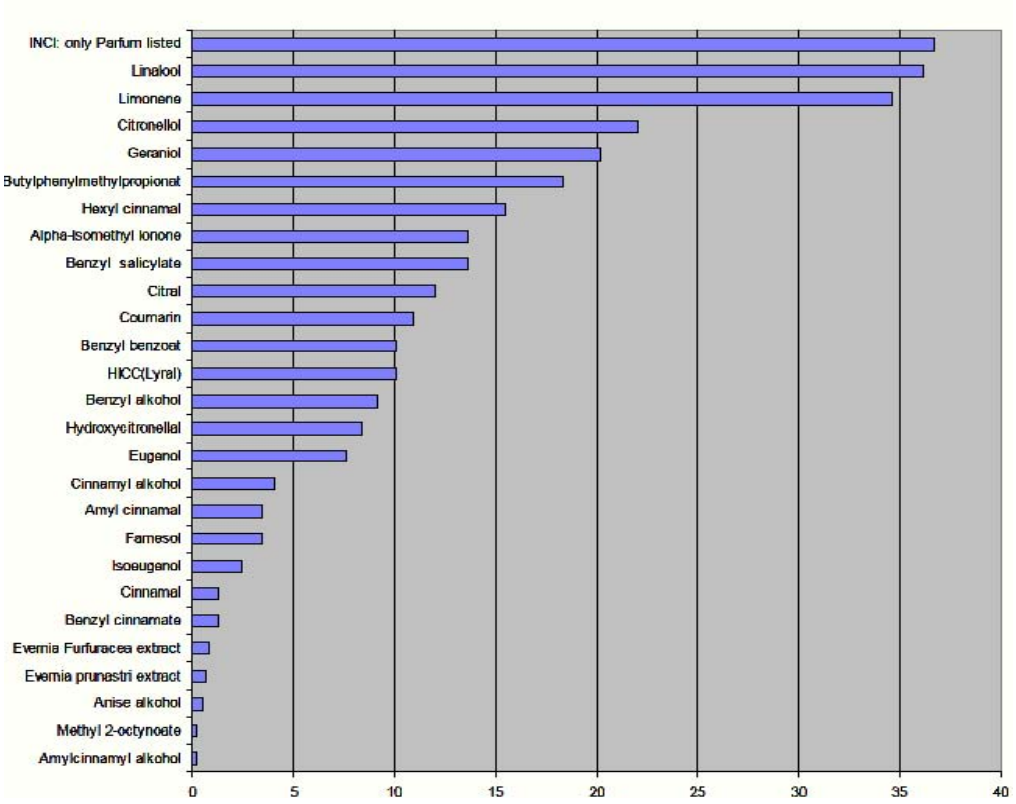


Figure 10-1: Frequency of occurrence in 3,000 consumer products of the 26 fragrance allergens that are required to be labelled in cosmetics and detergents (CVUA Karlsruhe, Germany, 2006/2007), according to (105).

Contents of fragrance substances determined in cosmetic products have been described in several studies, both before and after the regulation of the 26 fragrance allergens. The studies prior to the regulation of the 26 fragrance allergens included many, but not all of these 26 allergens. On the other hand, these studies included some other possible fragrance allergens. The quantitative analysis of fragrance substances has been performed in prestige perfumes (5, 157, 232-234), deodorants (228, 231), children's cosmetics and cosmetic toys (115, 227, 235), products marketed as natural cosmetics (225) and in cosmetics used by patients with contact allergy to fragranced products (35, 71). Quantitative analyses have revealed that the consumer is exposed to most, but not all of the 26 fragrance allergens from the use of cosmetics. However, when fragrance exposure from other consumer products, for example detergents and other household products is also taken into consideration (Table 10-3, Table 10-4, Figure 10-1), (105, 115, 229, 236), exposure to all of the 26 allergens is foreseeable in daily life. Although from the data available, the exposure to α-amylcinnamyl alcohol, cinnamal, methyl-2-octynoate, *Evernia prunastri* (oak moss) and tree moss may appear to be low, these are very strong allergens.

The changes in the use of fragrance chemicals in cosmetic formulations, during last 12 years, i.e. before and after the regulation of the 26 fragrance allergens, is reflected in the studies concerning contents of fragrances substances in popular perfumes (5, 232). As described in Table 10-5, the content of FM I allergens in prestige perfumes was significantly reduced from 1996 to 2003. Whether this is also the case for the perfumes sold as natural cosmetics (Table 10-6) has not yet been investigated.

Table 10-5: Concentration of Fragrance Mix I ingredients in five prestige perfumes before and after the regulation of the 26 fragrance allergens.

Fragrance INCI name	**Concentration in the perfumes before regulation (5)**			**Concentration in the perfumes after regulation (232)**		
	In no. of perfumes	**Range % (w/w)**	**Mean % (w/w)**	**In no. of perfumes**	**Range % (w/w)**	**Mean % (w/w)**
Geraniol*	5	0.072-0.432	0.340	5	0.090-0.236	0.156
Cinnamal	2	0.002-0.002	0.002	0	-	-
Hydroxy-citronellal	5	0.222-0.979	0.615	5	0.015-0.478	0.169
Cinnamyl alcohol	4	0.068-0.232	0.147	0	-	-
Eugenol	5	0.032-0.738	0.337	2	0.001, 0.001	0.001
Isoeugenol	3	0.026-0.249	0.119	2	0.001, 0.004	0.003
Amyl cinnamal	1	0.019	0.019	0	-	-

Note: * Due to interference by linalyl acetate, concentration of geraniol+linalyl acetate is reported.

Table 10-6: Concentrations of Fragrance Mix I ingredients, hexyl cinnamal and coumarin in 22 perfumes marketed as natural cosmetics investigated in 1996.

Fragrance	In no. of perfumes	Concentration % (w/w)
Geraniol	14	1.191*
Cinnamal	3	0.089, 0.109, 2.101
Hydroxycitronellal	5	0.135-6.044
Cinnamyl alcohol	8	0.035-2.289
Eugenol	2	0.027, 0.139
Isoeugenol	8	0.194-3.039
Amyl cinnamal	9	0.105-7.706
Coumarin	11	0.046-6.043

Note: * Quantification was performed in one sample only, due to interference by a very large amount of linalyl acetate in other samples.

The trend in the use of most of the fragrance allergens in deodorants before and after their regulation is reflected by the two studies performed by Rastogi et al. (228, 231). The results of these studies cannot be directly compared, because the study from 1998 included randomly selected deodorants, while selection of the deodorants for the 2007 study was based on the labelling of the presence of known strong fragrance allergens in these products. The number of products analysed in the 1998 study were three times more than those analysed in 2007, but not all of the 26 fragrance allergens were analysed in the 1997 study. However, an indication of the change in the use of the fragrance allergens during 1998-2007 may be obtained by reviewing the results of these two studies. Among the 17 common fragrance substances studied in the two studies, the frequency of use of 16 of these substances in deodorants was reduced in 2007 compared to that in 1998 (Table 10-2). The frequency of use of butyl phenyl methyl propional in deodorants appeared to be unchanged. The contents of benzyl alcohol, benzyl salicylate, cinnamal, cinnamyl alcohol, eugenol, geraniol, isoeugenol and linalool were found to be lower in the deodorants analysed in 2007 compared to those in 1998. Citronellol, coumarin and alpha-isomethylionone contents in the deodorants were similar in both studies, but concentrations of benzyl benzoate, butyl phenyl methyl propional, hexyl cinnamal, hydroxyisohexyl-3-cyclohexene carboxyaldehyde and linalool were much higher in deodorants in 2007 compared to those in 1998. This analysis of trend of use of fragrance allergens in cosmetic products indicates that the regulated fragrance allergens are used less frequently, but exposures from some of the regulated fragrance allergens may be much higher compared to those before regulation.

Table 10-7: Atranol and chloroatranol content in eau de toilette/eau de perfume, investigated in 2004 and in 2007.

	2007 Study	**2004 Study**
No. of samples	22	17
Atranol present in no. of samples	15 (68%)	12 (70%)
Atranol content	ppb (ng/ml)	ppb (ng/ml)
Range	n.d.-880	n.d.-791
Mean±SD	157±249	97±224
Median	47	20
Chloroatranol present in no. of samples	9 (41%)*	14 (82%)
Atranol content	ppb (ng/ml)	Ppb (ng/ml)
Range	0.9-208	1-175
Mean±SD	63±73	36±51
Median	22	10

Notes: n.d. Not detected.
*P <0.05 (chi-square test).
SD: Standard deviation.

Atranol (CAS no. 526-37-4) and chloroatranol (CAS no. 57074-21-2), constituents of oak moss and tree moss have been shown to be very potent fragrance allergens (237, 238). The EC Scientific Committee on Consumer Products (SCCP) recommended that atranol and chloroatranol should not be present in cosmetic products (239). Two other commonly used fragrance chemicals, isoeugenol (240) and hydroxyisohexyl-3-cyclohexene carboxyaldehyde (HICC) (71), have also been shown to be important contact allergens. The contents of atranol, chloroatranol, isoeugenol and hydroxyisohexyl-3-cyclohexene carboxyaldehyde in fine fragrances was determined for the exposure assessment of these fragrances (233). The results revealed that isoeugenol was present in 56%, HICC in 72%, atranol in 59%, and chloroatranol in 36% of the 22 eau de toilette/eau de parfum products. The concentrations of isoeugenol were, in all products, below 0.02% which is the maximum concentration recommended by the fragrance industry. HICC reached a maximum concentration of 0.2%, which is 10-fold higher than the maximum tolerable concentration considered safe by the EC Scientific Committee (241). The concentrations of atranol and chloroatranol in the products investigated in 2007 were comparable to those found in similar products in 2004 (Table 10-7, (233, 234). A significant decrease in the frequency of the presence of chloroatranol in the products was found in 2007 (Table 10-7).

10.2. Global exposure (household and occupational exposures)

Fragrances are used in cosmetics that the consumer applies to themself, as described in the previous section. In addition, exposure to fragrance substances is possible by a number of other exposure routes briefly outlined in this section.

Topical pharmaceutical products

In a study from Belgium, 370 of the 3,280 topical products marketed in Belgium have been found to contain one or more of 66 fragrance substances (242). This publication also contains a description of causative fragrance allergens in 127 patients reacting to 48 specific topical products. In a broader sense, exposure of the patient by extracts used in aromatherapy falls in this category as well.

Childrens products and toys

Children's products may contain fragrance allergens and high levels may be present (235). It has been stated that children may become sensitised to fragrance chemicals used by their mothers (243).

Clothing

Washed fabrics have been reported to contain fragrances (244). Odour-neutralising agents are sometimes used for shoe insoles. In one case, an insole containing cinnamon, has been reported to lead to plantar vesicular contact dermatitis due to contact sensitisation to FM I and, in the breakdown, to cinnamal and cinnamyl alcohol (245).

Cleaning agents and other household products

Contact dermatitis from geraniol in washing-up liquid has been reported (246). Terpenes are used as solvents and cleansing agents (e.g. limonene) (247) and have been reported as cause of hand dermatitits (248, 249). In an analysis of 59 household products the most common fragrance allergens were limonene (78%), linalool (61%) and citronellol (47%) (250). In a review of 301 cosmetic and detergent consumer products in Sweden, in half of the cosmetics and one-third of the detergents, one or more of the 26 fragrances requiring labelling were identified (251). In the UK, a review of 300 consumer products showed that linalool and limonene were present in 63% of products. Dental products contained on average 1.1 fragrance substances that are presently required to be labelled and women's perfumes contained 12 of these fragrance substances (Table 4-1 and Table 4-3) (229).

Candles

The dermal hand transfer of three fragrance materials (cinnamic aldehyde, d-limonene and eugenol) from scented candles was determined in ten subjects (i.e. 20 hands) after grasping scented candles for five consecutive 20 second exposures/grasps. The total mean residues of cinnamal and eugenol transferred per grasp from the candles to the hands were 0.255 µg/cm(2) and 0.279 µg/cm(2), respectively (252).

Food

Food causing cheilitis or bullous stomatitis (e.g. due to cinnamal (253)) or lichen planus-like lesions (e.g. due to cinnamal (254)) or contact gingivitis (e.g. due to eugenol (255)) has been reported. Moreover, food containing fragrance allergens, e.g. citrus oil terpenes (256) may cause allergic contact dermatitis by handling this food.

Occupational exposure

In a number of occupations, contact allergy to fragrances is more common than in others, including geriatric nurses, masseurs and physiotherapists, metal furnace operators and potters/glass makers, according to a multifactorial analysis (90). Moreover, hairdressers, beauty therapists and aroma therapists are examples of occupations where there is occupational exposure to fragrance-containing cosmetic and other products. Cleaners are exposed to fragrance-containing household products (e.g. detergents). Cooks and bakers are exposed to flavour chemicals and spices. Healthcare workers are also at risk of acquiring fragrance contact allergy. "Odour maskers" may contain important fragrance allergens (89, 90, 257-259). Occupational exposure and

occupational ACD to fragrances have been described in perfume bottlers (260). Industrial use of a powder masking the vinyl smell of car seats, containing cinnamal, causing occupational ACD has been reported (259).

A number of fragrance chemicals are also used as biocides (see Commission Regulation (EC) No 1451/2007 of 4 December 2007 on the second phase of the 10-year work programme referred to in Article 16(2) of Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market, published 11.12.2007 EN Official Journal of the European Union L 325/3 –L325/65), see Table 10-8 below.

Table 10-8: Parts of Annex I to (EC) No 1451/2007 (see above): "Active substances identified as existing", if use is 'perfuming' or 'masking' according to CosIng.

Biocide	EINECS	CAS number	Biocidal product group
Linalool	201-134-4	78-70-6	19
Geraniol	203-377-1	106-24-1	18, 19
Benzyl benzoate	204-402-9	120-51-4	2, 18
Eugenol	202-589-1	97-53-0	Not given
Farnesol	225-004-1	4602-84-0	Not given
(R)-p-mentha-1,8-diene	227-813-5	5989-27-5	12
Citriodiol/mixture of cis- and trans-p-menthane-3,8 diol	255-953-7	42822-86-6	1, 2, 19
Citral	226-394-6	5392-40-5	Not given
Pine ext.	304-455-9	94266-48-5	10
TANACETUM CINERARIIFOLIUM FLOWER EXTRACT	289-699-3	89997-63-7	18
Citrus oils (main component: limonene)	several	various	
Clove oil (main component: eugenol (83.8 %), caryophyllene (12.4 %)	/	8000-34-8	

Product groups(According to Biocide Directive 98/8/EC)

1 Human hygiene biocidal products
2 Private area and public health area disinfectants and other biocidal products
3 Veterinary hygiene biocidal products
10 Masonry preservatives
12 Slimicides
18 Insecticides, acaricides and products to control other arthropods
19 Repellents and attractants

The above illustrates that the consumer is exposed to fragrance substances from a wide variety of cosmetic products, other consumer products, pharmaceuticals and occupational exposures.

All these exposures are of importance in the context of contact allergy as it is not the source of exposure that is critical for both induction and elicitation, but the cumulative dose per unit area.

10.3. Exposures related to particular anatomical sites

Contact allergy to fragrances most often causes dermatitis of the hands, face and axillae. Axillary involvement has been shown to be statistically related to fragrance allergy (9). It is recognised that the axillary skin is a problematic area as it is moist, occluded and is easily irritated. Moreover, facial eczema is a common manifestation of fragrance allergy (3, 47). There is an association between fragrance allergy and hand eczema or aggravation of hand eczema (13-15). Vehicles may influence elicitation capacity of an allergen and the presence of detergents (surfactants) as in hand cleaning products may increase the clinical response by a factor of 4-6 (261). Men using wet shaving as opposed to electric razors have an increased risk of being fragrance allergic (17), most likely due to microtraumata and to the presence of surface active substances in shaving foam.

In use tests, the upper arm has been shown to be more sensitive than the forehead and lower arm (262). The axillae, neck and face are more sensitive than the upper arms (10). The threshold of elicitation may vary depending on the volatility of the substance (263). A cumulative effect of exposures occurs so that repeating exposures cause elicitation in more individuals (264).

Patients appear to become sensitised to fragrances primarily from deodorants and perfumes and to a lesser extent from other cosmetic types (74). Allergic contact dermatitis may develop where a perfume has been applied (behind ears, neck, upper chest, antecubital fossae, wrists and the axillae bilaterally (265). Following this, eczema may appear, or be worsened by, the use of a variety of product types including other cosmetics, household products, industrial products and flavours.

The association between contact allergy to fragrance ingredients and certain anatomical sites, which mirrors exposure to fragrance-containing products on these anatomical sites, has been described in several publications (266, 267), see above. However, due to the potential confounding effect of other factors, at least on some anatomical sites, an adjusted analysis will provide a more valid impression of the association between certain anatomical sites and contact allergy to fragrance ingredients. As an adjusted, multifactorial analysis relies on: (i) a substantial number of observations (patients tested); and (ii) an outcome prevalence not too close to 0%, such an approach has, hitherto, been limited to FM I.

In a paper published 2001, data from the IVDK in terms of patch test reactions to FM I and relevant clinical and demographic information of the patients tested (n=57,779) was studied by Poisson regression analysis (90). Risk was quantified by the prevalence ratio, which can be interpreted as an estimate of relative risk, i.e. the factor by which the risk of being sensitised to FM I (in this example) is to be multiplied (RR > 1: elevated risk; or RR < 1: reduced risk) if a certain “risk factor” is present, compared to those patients in whom this risk factor is not present (the reference category) (general aspects of such analyses are discussed in (268)). In the analysis, potential risk factors and confounders, respectively, including occupation, year of patch testing (to address a possible time trend), sex, age, past or current atopic dermatitis, in addition to anatomical site. The relevant part of Table 3 of (90) is reproduced below.

Table 10-9: Result of a Poisson regression analysis of patients tested with the Fragrance Mix between January 1992 and December 1998, considering two alternative outcomes – part I: non-occupational factors

Attribute	*Prevalence (%)*	*At least + (11.5%)*		*At least ++ (4.0%)*	
		PR	*95% CI*	*PR*	*95% CI*
Age:					
≤30	26.7	1.00	Reference	1.00	Reference
>30–44	23.8	1.42	1.31 to 1.53	1.61	1.40 to 1.84
>44–58	25.6	1.67	1.55 to 1.80	1.90	1.66 to 2.16
>58	23.9	1.93	1.77 to 2.10	2.07	1.79 to 2.39
Sex (female)	64.5	1.29	1.21 to 1.37	1.18	1.07 to 1.31
Main site:*					
Trunk	2.9	1.00	Reference	1.00	Reference
Hands	29.9	1.24	1.07 to 1.46	1.28	0.98 to 1.67
Arm	3.8	1.23	1.01 to 1.49	1.19	0.86 to 1.65
Face	15.2	1.20	1.03 to 1.42	1.13	0.86 to 1.48
Neck	1.4	1.39	1.10 to 1.75	1.31	0.88 to 1.94
Feet	2.8	1.26	1.02 to 1.55	1.19	0.84 to 1.68
Leg	8.7	1.59	1.36 to 1.89	1.50	1.14 to 1.99
Axilla	0.9	2.77	2.20 to 3.46	2.73	1.87 to 4.00
Other site	8.9	0.66	0.55 to 0.80	0.48	0.35 to 0.67

*Additionally controlled for several more sites—none of these associated with a significantly increased or decreased risk.

Compared to the trunk, which was arbitrarily chosen as the reference category, all other anatomical sites are associated with an increased risk of being sensitised to FM I (significantly if the lower limit of 95% CI is > 1). Most evidently, dermatitis of the axilla(e) is strongly associated with contact allergy to FM I, presumably due to the application of deodorants. Furthermore, the part of the table shown above illustrates a strong, positive age gradient, i.e. the older patients are, the more likely they are to be sensitised to FM I, the risk being almost double when comparing the oldest with the youngest age group. This observation is in concordance with a bivariate (unadjusted) association between age and contact allergy to FM I found in another study (89). This association is presumably the result of life long exposures and cumulative risk.

In a similar analysis of *Myroxylon pereirae* resin, published in 2002 (269): (i) an even stronger age gradient; and (ii) no particular association to axillary dermatitis (included in the "other" category) was found (Table 10-10).

Table 10-10: Association between selected risk factors and positive patch test to *Myroxylon pereirae* resin. For full model see (269). Risk quantified with the prevalence ratio (PR) with accompanying 95% confidence interval (CI).

Factor	**PR**	**95% CI**
Atopic dermatitis, past or present	1.02	(0.95-1.10)
Female sex	1.13	(1.06-1.20)
Site		
Trunk	1.00	(reference)
Hand or Arm	1.03	(0.94-1.12)
Foot or Leg	1.76	(1.61-1.92)
Head or Neck	0.94	(0.86-1.03)
"Other" site	0.72	(0.64-0.81)
Missing site	1.07	(0.97-1.19)
Age		
30 years and younger	1.00	(reference)
31 to 44	1.92	(1.73-2.12)
45 to 58	2.87	(2.61-3.16)
58 or older	3.85	(3.49-4.25)

10.4. Conclusion

There are various modes of exposure to fragrances, including not only products used for their scent, such as perfumes and eau de toilette, after shaves, and deodorants, but also types of products where scent is an added feature, such as other cosmetic categories (including wipes), topical pharmaceuticals, household products, and products encountered in the occupational setting.

Consumer exposure can change over time, both qualitatively and quantitatively.

Different routes of exposure are reflected by certain anatomical sites affected: deodorants are associated with axillary dermatitis, the axillary skin being particularly vulnerable to sensitisation due to occlusion, maceration and irritation. However, while sensitisation and initial disease may follow a distinct pattern, later less specific exposures, e.g. via hand creams, cleaning lotions etc. may be sufficient to cause allergic contact dermatitis.

11. Dose-response relationships and thresholds

The dose-response relationship between exposure to contact allergens and induction of allergy, i.e. sensitisation, is well established in animal models and by experiments in healthy volunteers (270). It seems that not only the dose per unit area of allergen (271), but also the number of exposures, i.e. the accumulated dose, is of importance for the risk of induction of contact allergy (272). The induction of contact allergy is an immunological process (type IV-allergy), which is without any clinical symptoms. In the case of continued exposure or re-exposure with a sufficient dose of allergen, elicitation will occur. Elicitation is an inflammatory response (eczema) with clinical symptoms of erythema, induration and in some cases vesicles. Studies of the elicitation response are normally done in patients with an allergy to the substance in question. Different provocation models exist (see chapter 11.2.1). Elicitation experiments in healthy human volunteers following the induction have only rarely been performed (273) and may be considered a less valid model than patient studies. The reason is that following experimental induction, the level of sensitivity may not be at the same level as in a real life situation and that individuals who have actually acquired the disease are a more relevant endpoint to study.

Knowledge of the dose-response relationship provides an opportunity to establish levels of exposure which are safe for the majority of individuals. In the following chapter, the use of different data and models for the establishment of such safe levels in relation to fragrance ingredients are explored. The focus will be on those chemicals, which have been identified in chapter 7.1 as established contact allergens in humans and which have already given rise to a significant number of published cases (category 3 or more): cinnamal, cinnamyl alcohol, citral, coumarin, eugenol, farnesol, geraniol, hydroxycitronellal, isoeugenol. Limonene and linalool are considered in chapter 5 as their ability to cause sensitisation depends on air oxidation, and hydroxyisohexyl 3-cyclohexene carboxaldehyde is considered in chapter 4.3.2 and 11.4.

11.1. Induction

A model for dermal sensitisation quantitative risk assessment (QRA) has been developed and implemented by the fragrance industry. This model relies on thresholds, no effect or low-effect levels, established in healthy human volunteers and/or in animal experiments, mainly the local lymph node assay (LLNA) (see chapter 8.1). A set of safety factors are applied for inter-individual differences, for vehicle effects and for use considerations, stated to give rise to a safety margin from 10 to 1000 (274). In this way, a so-called “acceptable exposure level” is derived. The exposure to an allergen in different types of products should be below this level. The restrictions, which have been introduced by the fragrance industry based on the QRA model, are given in Table 11-1 for some important product categories.

The IFRA guidelines give concentration limits for 11 product categories (http://www.ifraorg.org/en-us/standards_1, last accessed 2011-11-02), three of which are mentioned in Table 11-1. These three products have the lowest concentrations except for lip products, which give a slightly lower concentration limit.

Table 11-1: Current IFRA restrictions based on induction experiments.

Fragrance chemicals	IFRA guideline[1]		
	Deodorant (%)	Hand cream (%)	Perfume (%)
Cinnamal	0.02	0.05	0.05
Cinnamyl alcohol	0.1	0.4	0.4
Citral	0.05	0.3	0.6
Coumarin	0.13	0.8	1.6
Eugenol	0.2	0.5	0.5
Farnesol	0.11	0.6	1.2
Geraniol	0.4	2.8	5.3
Hydroxycitronellal[2]	0.2	1.0	1.0
Isoeugenol[2]	0.01	0.02	0.02

Notes: 1) Exposure per mg/cm^2/day is based on 8.5 mg/cm^2/day for deodorants, 2.2 for perfumes and 4.2 for hand creams as it is these exposure levels that are used by the IFRA.
2) Cosmetic Directive Annex III: Hydroxycitronellal restricted to 1% in all products and isoeugenol to 0.02% in all products.

The SCCP evaluated this methodology (275) as well as its application to three model fragrance substances.

It was, among other things, concluded that:

> "The data provided show that the application of the dermal sensitisation QRA approach would allow increased exposures to allergens already known to cause allergic contact dermatitis in consumers. The model has not been validated and no strategy of validation has been suggested. There is no confidence that the levels of skin sensitisers identified by the dermal sensitisation QRA are safe for the consumer."

and that:

> "Identification of safe levels of exposure to existing substances known to cause allergic contact dermatitis in the consumer should be based on clinical data and/or elicitation low-effect levels. Currently, these are the only methods which have proven efficient in reducing/preventing existing problems of sensitisation/allergic contact dermatitis in the consumer."

11.2. Elicitation

11.2.1. General considerations

A response in terms of elicitation of allergic contact dermatitis by application of the (suspected) allergen under standardised conditions is the outcome of interest of the routine diagnostic procedure for suspected contact allergy, the patch test. While the patch test procedure is largely standardised, it is optimised as a diagnostic tool for contact allergy. Thus exposure conditions are not comparable to actual exposures occurring in the daily life or working environment of the patient, which often involve long-term, repeated and low-dose contact with the allergen. Here, procedures such as the repeated open application test (ROAT) or provocative use test are often used, because they reflect actual exposure much better and can be used, for instance, to validate the current clinical relevance of a positive PT reaction.

Generally, exposure of a sensitised patient to a set of graded doses (quantity/area) of the suspected allergen, i.e. threshold testing, will allow not only quantitative diagnosis of the presence or absence of specific contact sensitisation but will additionally provide evidence on the intensity (degree) of sensitisation. This may have important individual consequences in terms of everyday or occupational exposures being capable (or not) of eliciting allergic contact dermatitis. However, beyond the individual perspective, clinical dose-response data collected from sensitised individuals provide a valuable estimate of the usual doses/unit area resulting in a positive, allergic response in a certain proportion of sensitised persons, e.g. 10, 50 or 90%. Maximum concentration levels can be derived, which are safe in terms of eliciting allergic reactions in only a defined low percentage of sensitised persons. As such data will always be based on small samples, the precision of the estimate should be considered, and therefore results are preferably given with confidence intervals.

A statistically significant relationship between threshold concentrations in the ROAT and patch test has been found, on analysing results from different allergens (see Table 11-2) (276), but the dose of allergen per unit area per application needed to elicit a reaction in the two study methods is not the same. A translation factor between the two methods has been suggested for non-volatile substances: EDxx(ROAT)=0.0296 *EDxx(patch test) based on testing nickel and methyldibromo glutaronitrile (276). Based on this the eliciting dose per application in an open test is 33 times lower than in the patch test. In practice it means that the cumulative dose in a ROAT (in µg/cm²) in two weeks with two applications per day (total 28 applications) will be almost identical to the eliciting patch test dose (in µg/cm^2) for a given number of responders (see Figure 11-1). For a given cut-off point the elicitation dose determined by patch testing will be higher than determined by ROATs.

Table 11-2: Spearman's rank correlation between the threshold concentration in the patch test and the repeated open application test for three allergens.

Allergen	Number of patients	Correlation coefficient	*P*-value
Nickel	18	0.45	0.033
MDBGN	15	0.76	0.0021
HICC	16	0.59	0.011

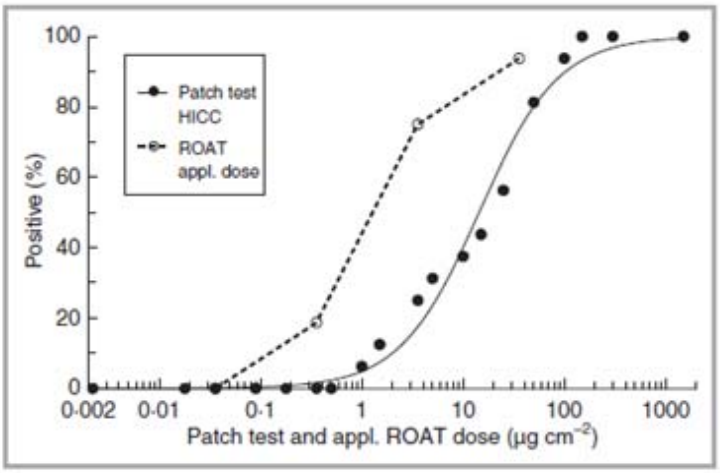


Figure 11-1: The fitted dose-response curve for patch test (solid line) is seen to be displaced to the right compared to the observed response from repeated open applications of the same allergen (HICC). It means that a smaller dose per application is needed to elicit a response than by one single occluded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

In the translation between methods, evaporation needs to be taken into consideration for volatile substances. The experience, based on a study of the fragrance ingredient HICC and using the results from the literature on isoeugenol, is that if the same equation is used as for non-volatile substances, the response in the ROAT will be overestimated by a factor 3 to 4. Thus, the translation factor would be 0.1060 instead of 0.0296, but this needs to be confirmed by other fragrance allergens. This implies that for the fragrance ingredients tested, the eliciting dose per application in a ROAT was 9.4 times lower than the patch test compared to a 33 times lower dose for non-volatile substances (276). This needs to be confirmed by studying other fragrance allergens. Thus, according to these experiments, the dose ($\mu g/cm^2$) eliciting a response in threshold patch testing will be at most 33 times higher than established in the ROAT if an identical vehicle is used.

Volatility effects in skin sensitisation

The potency of volatile skin sensitisers can be underestimated, to an extent depending on how rapidly it evaporates, by assays such as the LLNA in which the test substance is applied topically to exposed healthy skin without occlusion. Such sensitisers present a greater sensitisation risk to consumers when the skin is occluded by clothing and/or compromised, than when healthy non-occluded skin is exposed.

Volatility at physiological temperature, say 40˚C, is represented by the vapour pressure p_{40} at that temperature. This is related to the boiling point T_B by the Clapeyron-Clausius equation, which can be written (277):

$$\text{Log } (p_{40}) = - (T_B - 40)Tr/2.303RT$$

Where p is in atmospheres, T_B is in ˚C, R is the gas constant, Tr is the Trouton constant (also defined as the molar entropy of vaporisation, and equal to 22 $cal.deg^{-1}$ for many organic compounds) and T is physiological temperature in degrees absolute (= 313 for 40˚C).

It has been shown, in experiments where evaporation from a glass slide is measured under simulated LLNA conditions, that 2-hexenal (T_B = 146-149˚C, p_{40} = 17 mmHg) evaporates rapidly, less than 20% remaining after 5 minutes, whereas with cinnamal (T_B = 248˚C, p_{40} = 0.5 mmHg), more than 90% remains after 1 hour (278). In agreement with these findings, cinnamal fits a QSAR relating LLNA EC3 to reactivity, whereas the EC3 for 2-hexenal is higher (lower potency) than predicted from its reactivity.

The above is only a partial rationalisation, since different solubilities in different vehicles will influence the tendency to evaporate, according to Henry's law.

11.2.2. Studies on specific fragrance ingredients

Studies concerning chloroatranol/atranol, cinnamal, hydroxycitronellal, hydroxyisohexyl 3-cyclohexenecarboxaldehyde and isoeugenol have been identified. These are summarised in Annex III.

Overview of results

In four studies dummy deodorants spiked with a single fragrance allergen in realistic use concentrations have been used to study elicitation responses, unscented deodorants were used as control products in paired designs. The deodorants were used by patients sensitised to the fragrance allergen in question as well as a healthy control group

(without fragrance allergy) (102,103,104,279). Between 76 and 100% of the sensitised individuals reacted to the deodorants spiked with allergen, isoeugenol, cinnamal, hydroxycitronellal and hydroxyisohexyl 3-cyclohexene carboxaldehyde, and none of the controls (Table 11-4).

Table 11-3: Overview of results of deodorant provocation investigations with different allergens. Frequency in % of test groups, which reacted at different doses of allergen applied in a roll-on deodorant in the axilla, is given in the table.

Dose in ppm in deodorant	**Isoeugenol**	**Cinnamal (1)**	**Cinnamal (2)**	**Hydroxycitronellal**	**HICC**
0	0	0	0	0	0
63	23				
100			11		
200	69				64
320		25	55	57	
600					85
630	76				
1000		75	88	71	
1800					100
3200		100		100	
No. test persons	13	8	9	7	14
No. of control persons	10	20		7	10
% control persons, who reacted	0	0		0	0
Exposure according to study should be:	**< 63 ppm**	**<100 ppm**		**<320 ppm**	**< 200 ppm**
Reference	(279)	(103)		(104)	(102)

Note: HICC hydroxyisohexyl 3-cyclohexene carboxaldehyde.

Eleven studies concerning dose-response results of the five allergens listed above were identified, including the above mentioned studies of deodorants. An overview of the results of the studies concerning thresholds is given in Table 11-4. In Annex III the details of each study are given.

Table 11-4: Overview of threshold results from clinical studies.

"Observed" means that the proportion was actually observed in the study while "estimated" means that the value is derived from a fitted curve, i.e. is interpolated.

Chloroatranol			
ROAT			**Ref.**
In ethanol 92 % positive	0.025 µg/cm^2	observed	(238)
In ethanol 100% positive	0.125 µg/cm^2	observed	(238)
PATCH TEST			

ED10%	0.0004 µg/cm^2	estimated	(238)
ED50%	0.0045 µg/cm^2	estimated	(238)
Cinnamal			
ROAT			
In ethanol no effect	0.02%	observed	(101)
In ethanol 44 % positive	0.1%	observed	(101)
In ethanol 72 % positive	0.8%	observed	(101)
Deodorant matrix 11% positive	0.26 µg/cm^2 (0.01%)	observed	(103)
Deodorant matrix 41% positive	0.84 µg/cm^2 (0.032%)	observed	(103)
Deodorant matrix 82% positive	2.63 µg/cm^2 (0.1%)	observed	(103)
PATCH TEST			
ED50%	96 µg/cm^2	estimated	(101)
No effect level	0.4 µg/cm^2 (0.01%)	observed	(101)
No effect level	NG (0.002%)	observed	(103)
HICC			
ROAT			
In a cream base ED10%	4.9 µg/cm^2	interpolated	(105)
In a perfume (ethanol) ED10%	1.2 µg/cm^2	interpolated	(105)
In ethanol 61% positive	15.3 µg/cm^2 (3.4-22.2)	observed	(224)
In ethanol 89% positive	126.2 µg/cm^2 (40.5-226.2)	observed	(224)
In ethanol/water no response	0.0357 µg/cm^2	observed	(263)
In ethanol/water ED10%	0.064 µg/cm^2	estimated	(263)
In deodorant matrix between 64% to 100% positive	0.79 µg/cm^2 (median)	observed	(102)
PATCH TEST			
ED10% (95% CI)	0.662 µg/cm^2 (0.052-2.35)	estimated	(263)
ED10%	0.75 µg/cm^2	estimated	(102)
ED10%	0.9 µg/cm^2 29 (7-69) ppm	estimated	(224)
ED50% (95% CI)	11.1 µg/cm^2 (3.41- 33.1)	estimated	(263)
ED50% (95% CI)	18.3 µg/cm^2 (3.41- 33.1)	estimated	(102)
ED50% (95% CI)	20 µg/cm^2 662 (350-1250) ppm	estimated	(224)
No effect level	<0.0022 µg/cm^2	observed	(263)
Hydroxycitronellal			
ROAT			
Deodorant matrix 57 % positive	0.94 µg/cm^2 (0.032%)	observed	(104)
Deodorant matrix 71 % positive	2.94 µg/cm^2 (0.1%)	observed	(104)
Deodorant matrix 100 % positive	9.40 µg/cm^2 (0.32%)	observed	(104)
PATCH TEST			

No effect level	<0.00012 % (=0.036 µg/cm^2)* (*calculated)	observed	(104)
Isoeugenol			
ROAT			
in ethanol 63% positive	5.6 µg/cm^2	observed	(100)
in ethanol 42% positive	2.2 µg/cm^2	observed	(264)
in ethanol 67% positive	9.0 µg/cm^2	observed	(264)
Deodorant matrix 23 % positive	0.167 µg/cm^2	observed	(279)
Deodorant matrix 69 % positive	0.53 µg/cm^2	observed	(279)
Deodorant matrix 77 % positive	1.67 µg/cm^2	observed	(279)
PATCH TEST			
ED50% (in petrolatum)	32 µg/cm^2	estimated	(100)
No effect (in ethanol)	<0.0005% (0.15 µg/cm^2)	observed	(264)
No effect (in petrolatum)	<0.4 µg/cm^2	observed	(100)

Summary of results for specific fragrance ingredients

Chloroatranol (constituent of *Evernia prunastri*)

In ROAT a dose of 0.025 µg/cm^2 to 0.125 µg/cm^2 in ethanol elicited reactions in 92% to 100% of sensitised subjects.

In patch testing the ED10% was 0.0004 µg/cm^2.

Cinnamal

In ROAT a dose of 0.26 µg/cm^2 gave a response in 11% when applied as deodorant in the axilla and 82% responded to 2.63 µg/cm^2.

The ED50 in patch testing was 96 µg/cm^2.

HICC

In ROAT a dose of 0.0357 µg/cm^2 gave no response, while the dose that elicited a reaction in 10% of the sensitised test group (in ethanol) ranged from 0.064 µg/cm^2 to 1.2 µg/cm^2. The dose in a cream base was 4.9 µg/cm^2.

In ROAT a dose of 15.3 µg/cm^2 to 126.2 µg/cm^2 in ethanol elicited reactions in 61% to 89% of sensitised subjects.

The ED10 in patch testing ranged from 0.66-0.9 µg/cm^2.

Hydroxycitronellal

In ROAT a dose of 0.94 µg/cm^2 gave a response in 57% when applied in a deodorant in the axilla and 100% responded to 9.40 µg/cm^2.

The no-effect level in patch testing was below 0.036 µg/cm^2.

Isoeugenol

In ROAT a dose of 2.2 µg/cm^2 a response in 42% and 9.0 µg/cm^2 in 67%, when applied in ethanol on the arm. With a deodorant applied to the skin of the axillary, a dose of 0.167 µg/cm^2 caused a response in 23% and 77% reacted to 1.67 µg/cm^2.

The ED50 in patch testing was 32 µg/cm^2.

The no-effect in patch testing was below 0.15 µg/cm^2.

Elicitation levels have been studied for cinnamal, isoeugenol and hydroxycitronellal which are established contact allergens in humans and which already have given rise to a significant number of cases (> 100, see chapter 7). Further HICC has been studied extensively, but is considered in a separate section (chapter 11.3) of this opinion. It is however not possible to derive a safe threshold directly from the data of cinnamal, isoeugenol and hydroxycitronellal. The main reasons are that many of the test subjects reacted to all the tested doses in ROAT, which is a simulation of every day exposures. Thus it was not possible to determine the dose only eliciting responses in a few, e.g. 10% of the subjects and that only a limited number of exposure scenarios were studied.

The studies have covered few product types: hydro-alcoholic products, e.g. perfumes and deodorant roll-on matrix. The vehicle is one of many factors which influence the thresholds of allergic reactions. Also the presence of irritants and other allergens can influence the elicitation level. This means that the currently available studies do not cover all the relevant exposure scenarios. However, taking into account that dose-response investigations in sensitised patients are very complex to perform, it is not likely that much more data will become available in the near future. It is therefore necessary to exploit the full pool of elicitation data, also covering chemicals other than fragrance ingredients, to derive a more general threshold which could be used when no or insufficient data exist to set a specific threshold for a substance of concern.

General thresholds

The methodology of the different experiments has varied to some extent as different anatomical sites of exposure have been employed, different vehicles, exposure periods and cut-off points. The reason is that the studies have been performed to investigate various clinical and scientific aspects of allergic contact reactions and not for formal regulatory requirements. Some studies are small and for this reason the precision of the estimates of thresholds is limited. In spite of this, the results of the various experiments are reasonably uniform, except for chloroatranol which had very low threshold reactions, and show that low concentrations may elicit allergic reactions.

The reasonably uniform data generated on the above fragrance ingredients are in agreement with a recent “meta-analysis” of dose-response data of different allergens, incorporating some of the same studies as mentioned above, but also other allergens, such as preservatives and metals. The ED10 at patch testing varied by a factor of 7 from the lowest to the highest value and the median was 0.82 µg/cm^2 if the three outliers formaldehyde (1997), nickel (1999) and methyldibromo glutaronitrile (2004) were left out and 0.84 µg/cm^2 if included (see Table 11-6 and Figure 11-2 below: (280)). An explanation of these results could be that thresholds in elicitation is less dependent on the antigenic properties of the individual substance (inherent potency) than thresholds of induction and more on the level of sensitivity of the individual, i.e. the level of T-cell clones able to recognise the antigen, which is not present in naïve not-sensitised, individuals. This seems plausible, based on both the recent clinical evidence (280) and guinea pig QSAR evidence (281). It provides the basis for a general approach in establishing safe thresholds for substances of concern.

The consequences of a limit of 0.8 µg/cm^2 for the product types most important for fragrance allergy are calculated below.

The calculation is based on:

- The generally safe exposure level, which is the median ED10 value (the dose which will elicit allergic contact dermatitis in 10% of sensitised eczema patients) under patch test conditions: 0.8 µg/cm^2 (280).

- Exposure doses and exposure areas from SCCS notes of guidance 7th revision (282) [Tables 2 and 3] and Technical dossier Quantitative Risk Assessment from RIFM (274).

Equation:

Safe concentration in product = (Generally safe exposure level (0.8 µg/cm^2)/daily exposure to product (µg/cm^2/day)) x 100 (for %).

Table 11-5: Concentration limits in different product types based on 0.8 µg/cm^2 allergen as a 'generally safe exposure level', if specific dose-response data are unavailable.

	Estimated daily exposure level (g) (Table 3 SCCS NoG)	**Mean exposed skin surface (cm^2) (Table 2 SCCS NoG)**	**Exposure /cm^2/day in grams**	**Exposure /cm^2/day in µg (1g= 1x10^6 µg)**	**Concentration limit in product % in product: (GEL/daily exposure) x 100**
Body lotion	7.82 g	15,670 cm^2	0.000499	499	0.16%
Face cream	1.54 g	565 cm^2	0.002725	2725	0.03%
Hand cream	2.16 g	860	0.002511	2511	0.03%
Deodorant aerosol spray ethanol based	1.43 g	200 cm^2	0.007150	7150	0.01%
Perfume spray	not given	?	0.00221[1)]	2210	0.04%

Note: 1) 2.21 mg/cm^2/day from Technical dossier Quantitative Risk Assessment.

The estimated daily use of the various product categories in Table 11-5 are based on the SCCS Notes of Guidance (see above), except for perfume, for which no value is given. This value is taken from the Technical Dossier on Quantitative Risk Assessment from RIFM.

Generally the estimated use of different products is higher in the IFRA/RIFM assessments than in SCCS Notes of Guidance.

Table 11-6: Overview of dose-response studies and thresholds for eight allergens, after (280).

ED10 patch test values from each of the 16 selected studies with 95 % confidence intervals with the allergens chromium (283), MCI/MI (Kathon ™ CG) (284), nickel (285), methyldibromo glutaronitrile (MDBGN) (286), hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) (102, 224, 263), isoeugenol (264, 279) and formaldehyde (287). The shaded values were considered as outliers.

Study	**Number of patients**	**ED_{10} (µg/cm^2)**	**95 % interval**
MCI/MI	12	1.05	0.17–2.27
Formaldehyde	20	20.1	4.09–43.9
Nickel 1997	24	1.58	0.32–4.04
Nickel 1998	19	0.8	0.078–2.59

Study	Number of patients	ED_{10} (µg/cm^2)	95 % interval
Nickel 1999	26	7.49	2.42–14.5
Nickel 2005	13	0.74	0.066–2.38
Nickel 2007	20	0.82	0.13–2.37
Cobalt 2005	11	0.44	0.033–1.3
Chromium	17	1.04	0.0033–5.55
Isoeugenol 2001	24	1.48	0.22–4.74
Isoeugenol 2005	13	0.23	0.0073–1.32
HICC 2003	18	0.85	0.062–3.26
HICC 2007	14	1.17	0.043–5.05
HICC 2009	17	0.66	0.052–2.35
MDBGN 2004	19	0.025	0.00021–0.19
MDBGN 2008	18	0.50	0.052–1.69

Note: The ED10 value is the concentration which elicits an allergic reaction in 10% of a group of sensitised individuals under patch test conditions.

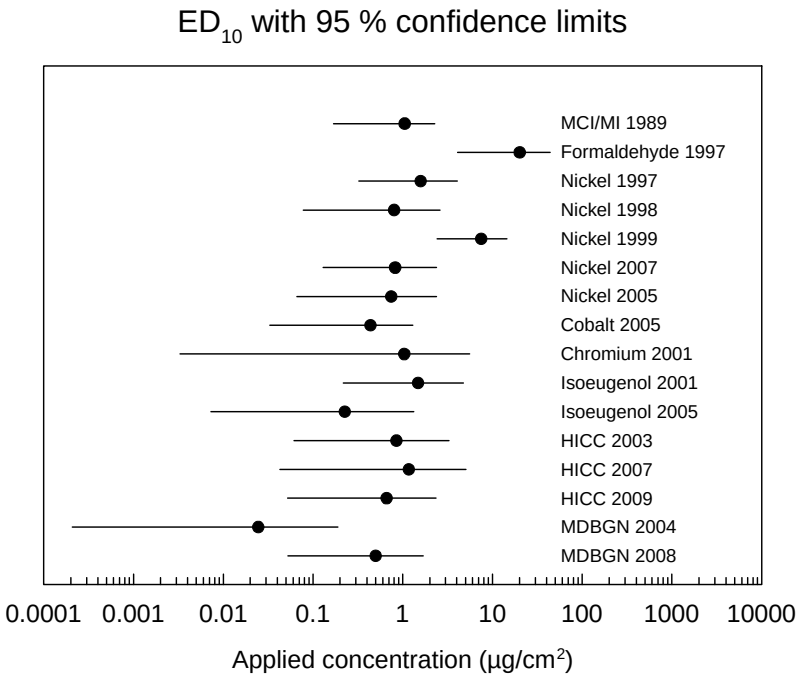


Figure 11-2: The threshold data with 95% confidence intervals from Table 11-6 presented graphically, after (280).

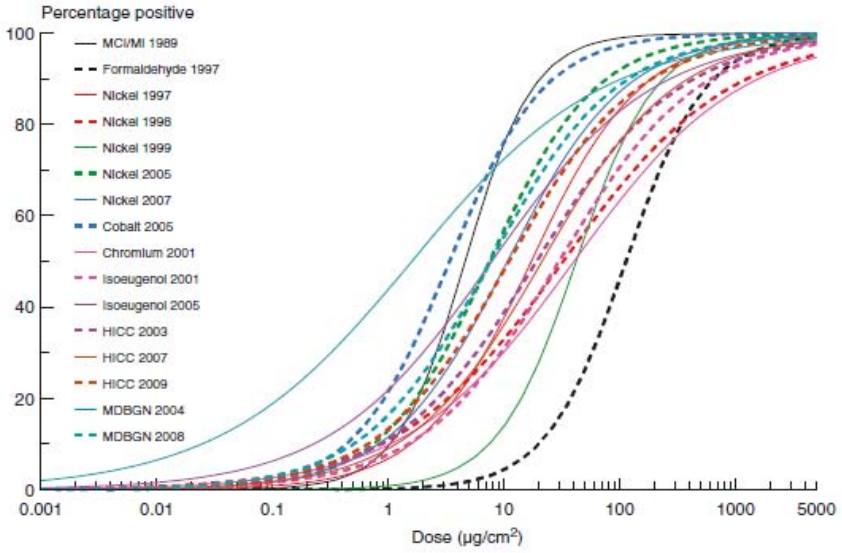


Figure 11-3: The fitted dose-response curves from the studies in Table 11-6**,** which are the basis for estimation of the ED10 value, after (280).

The meta-analysis above has shown that the median elicitation dose by patch testing for 10% of sensitised individuals was 0.8 µg/cm^2. In the model data for the fragrance substances isoeugenol and HICC was included. The two studies on isoeugenol and the three studies on HICC gave an average ED10 value of 0.85 µg/cm^2 and 0.89 µg/cm^2 with a range 0.23-1.48. This means that even if the model was used for these substances individually the result would be very similar to the general threshold value.

The data from cinnamal and hydroxycitronellal studies was not incorporated in the model because: (i) serial dilution patch testing was done in petrolatum for cinnamal, making the dosing less exact; (ii) and only seven patients participated in the hydroxycitronellal study, while a criteria for inclusion in the model was ten participants (280).

According to the above calculations, a limit of 0.8 µg/cm^2 for the product types of most importance for fragrance allergy corresponds to concentrations of 100 to 400 ppm (0.01-0.04%) for deodorants, perfume spray, hand and face lotions. For body lotion the general threshold was 0.16%. However, it does not seem meaningful in the context of contact allergy to distinguish between different types of creams, as a body cream would be applied with the hands and the relevant parameter in contact allergy is dose per area skin and not total dose.

A general threshold would have to take into consideration the uncertainties in quantification of exposure and safe thresholds as well as the possibilities of aggregate exposures and exposure to chemically similar substances. Therefore in setting one general threshold the product category carrying the highest risk of sensitisation and elicitation, which is deodorants, was chosen to drive the generation of the threshold. This means that a threshold of 0.8 µg/cm^2 is equal to 0.01% or 100 ppm (see Table Table 11-1 and the related text), the lowest of the threshold values derived.

The approach taken by the SCCS is based on scientific evidence published in peer-reviewed journals (283)(284)(285)(286)(102, 224, 263)(264, 279)(287) in the past 20 years. The meta-analysis deriving the general threshold limit at 0.8 ug/cm² limit has been published (280) in a peer-reviewed journal. The use of threshold limits based on elicitation data is a well established methodology which has been applied (with success) in EU to prevent further cases of induction and elicitation (primary and secondary prevention) in the case of nickel allergy, chromium in cement, chromium in shoes in

Germany, dimetyl fumurate in consumer items and also in part in IFRA guidelines e.g. concerning HICC.

The elicitation threshold model is based on 16 studies of 8 allergens, two of which are fragrance ingredients. It includes data from moderate to extreme allergens with a median EC3 value of 1.2.

The 11 fragrance allergens to which the limit is suggested to apply range from extreme to moderate with median EC3 value of 4.8, although in the case of coumarin an EC3 value could not be established.

Thus in general the potency profile of the fragrance substances of concern is not very different from those included in the model to provide the suggested general safe threshold.

The approach is targeting the relevant end-point, namely, allergic contact dermatitis. The mere consideration of potency of the allergen, according to the LLNA (EC3), is insufficient in identifying the size of the problems of contact allergy/allergic contact dermatitis. Additional information is needed from clinical and epidemiological studies, exposure assessment and dose-elicitation studies. For instance, the elicitation thresholds of e.g. HICC (EC3: 17.1) and isoeugenol (EC3: 0.54) are very similar (0.85 µg/cm² and 0.89 µg/cm², respectively) despite very different potencies. Both are frequent causes of contact allergy.

It should be noted that the general threshold is only suggested to be used for substances of concern if no specific data of sufficient quality exist to set an individual safe threshold. In cases where specific data of sufficient quality are available, these data should be used to set an individual safe threshold.

The general threshold is indicative of a safe level for the majority of sensitised individuals, but does not preclude that the most sensitive subset of the population may react upon exposure to the allergen. These levels are based on patch tests and take no account of anatomical sites of exposure, frequency of exposure or vehicle effects. Therefore, any limitations in exposures are not substitutes for providing information to the consumer about the presence of a substance in a product as a certain fraction of sensitised individuals will still need to avoid specific exposures.

Based on experience, limitations in exposure based on elicitation thresholds will, apart from helping the sensitised consumer, also significantly reduce the risk of induction. This is the case for nickel allergy, where the restrictions in the EU nickel directive are based on elicitation threshold, leading to a significant reduction in new cases of sensitisation in young women (288) and in a reduction in morbidity, i.e. elicitation (289). Another example is restriction of chromium VI in cement (290).

It is not possible to provide a safe threshold for natural extracts of concern, as no specific investigations exist, and the model providing the general use concentration limit (0.01%) has been based on chemicals only.

The SCCP concluded in 2004 that Chloroatranol and atranol, the main allergenic constituents of *Evernia prunastri and Evernia furfuracea*, should not be present in consumer products because they are extremly potent allergens (239). The persistently high frequency of contact allergy to *Evernia prunastri* and *Evernia furfuracea* noted in eczema patients does point to a persisting problem with exposure to the allergenic constituents.

11.3. Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) has been the most frequently reported individual fragrance chemical causing allergy since the 1999 opinion on fragrance allergy. In total, reports of about 1500 cases have been published in the scientific literature (see chapter 7.1 and Annex I to this opinion), while the second most

frequently reported individual chemical was cinnamal with around 350 published cases. Only a minority of the cases seen by clinicians is published and only a (small) proportion of those with allergic contact dermatitis seeks or has the possibility to seek medical attention.

Natural extracts such as *Myroxylon pereirae* and turpentine (oil) have been more frequently reported, but while HICC is a synthetic fragrance chemical, where the only source of exposure is fragrances, the natural extracts are used in many other contexts than fragrances/cosmetics.

Of patients tested by the Danish monitoring network of dermatologists 2.4% were found to be allergic to HICC in 2005-2008 (with no decreasing trend from 2003 to 2007 (291)) (for more studies see chapter 4.3.2); in 70% of the cases the reaction was of current relevance, i.e. causing disease (69). This is in agreement with the results of a recent German study with HICC, where 48 out of 51 patients (94.1%) with a positive patch test reaction to HICC also reacted in a repeated open application test, simulating normal use conditions of cosmetics containing HICC (105). In a Danish study 69% of 14 HICC allergic individuals developed allergic contact dermatitis from use of cosmetics containing HICC in realistic amounts (102).

On the basis of the high frequency of allergy to HICC, in 2003 the Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) recommended 0.02% (200 ppm) as maximum amount of HICC in cosmetic products (292). This has not been implemented and no restrictions apply in the Cosmetic Directive.

The fragrance industry, via the International Fragrance Association (IFRA), has its own safety guidelines. Up until 2003 HICC was used without any restriction; in 2003 a limit of 1.5% HICC in any kind of product was introduced. In 2008 this was changed according to the new risk assessment model (QRA) applied by the fragrance industry to different levels in 11 different product types derived from the QRA (see11.1). Limits from 0.11% in lip products to 1.5% in hair styling products were set. In 2009 a further lowering was made of the limits by industry with the following reasoning: “The industry firmly believes and continues to support thresholds based on induction rather than elicitation. However, given the exceptional situation in Europe, the fragrance industry elected to take further restrictive action on this material” (293). An overview of the IFRA restrictions is given in the table below.

Table 11-7: Restriction for HICC independent of the QRA according to (293).

IFRA QRA Category	Product type that drives the category	Consumer exposure level 2003–2008 (%)	IFRA Standard July 2008 (%)	IFRA Standard July 2009 (%)
Category 1	Lip products	1.5	0.11	0.02
Category 2	Deodorants/ antiperspirants	1.5	0.15	0.02
Category 3	Hydroalcoholics for shaved skin	1.5	0.60	0.2
Category 4	Hydroalcoholics for unshaved skin	1.5	1.5	0.2
Category 5	Hand cream	1.5	1.0	0.2
Category 6	Mouthwash	1.5	1.5	Not applicable*
Category 7	Intimate wipes	1.5	0.3	0.02

Category 8	Hair styling aids	1.5	1.5	0.2
Category 9	Rinse-off hair conditioners	1.5	1.5%	0.2%
Category 10	Hard surface cleaners	1.5	1.5%	0.2%
Category 11	Incidental or non-skin contact	15	Not restricted	Not restricted

Note: HICC Hydroxyisohexyl 3-cyclohexene carboxaldehyde.
QRA Quantitative risk assessment.
* Not applicable because HICC is not approved for flavour use.

As an update since the presentation of the pre-consultation version of the opinion, surveillance data on HICC from two European countries have become available, covering the period 2002-2011 (IVDK/Germany (294)) and 2003-2011 (Danish contact dermatitits group (295)), respectively. The first analysis identified a slight decrease, which was considered “not overwhelming in absolute terms”, namely, from 2.3% in 2002 to 2.1% in 2011 (crude prevalences, Figure 11-4). Thus, despite statistical significance, the decrease is too slight to be interpreted as relevant improvement. In the Danish study, some fluctuation around a mean prevalence of about 2.5% was noted, but no trend (Figure 11-5). It is reported that 74% of the positive reactions were regarded as clinically relevant.

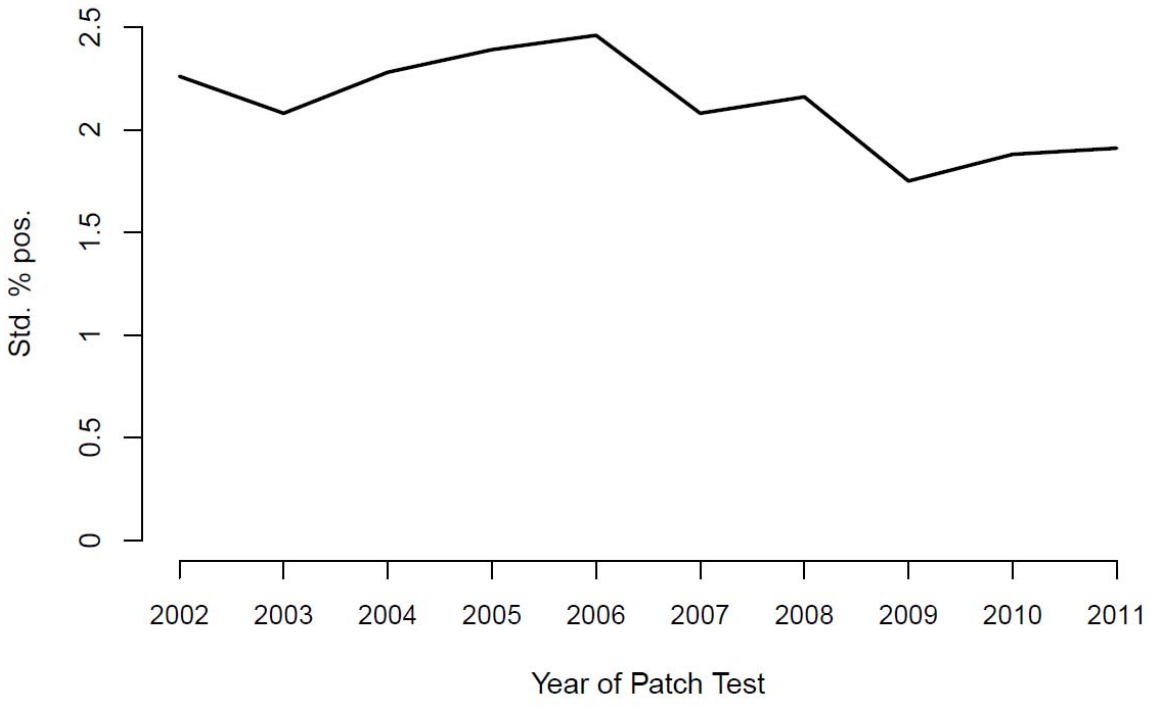


Figure 11-4: Time trend of hydroxyisohexyl 3-cyclohexene carboxaldehyde sensitisation prevalence [standardised prevalence of positives (%)] during 2002-2011. The decrease over time is statistically significant, after **(294)**.

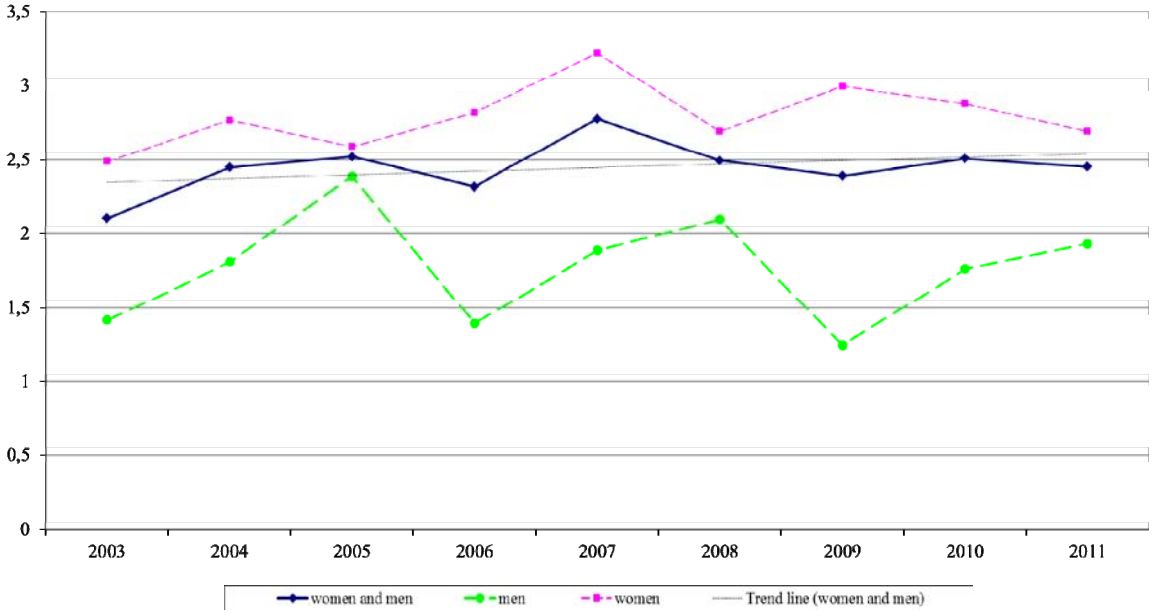


Figure 11-5: Prevalence of positive patch test reactions to hydroxyisohexyl 3-cyclohexene carboxaldehyde over time in 37 860 subjects tested by the Danish Contact Dermatitis Group (295).

11.4. Conclusion

- A dose-response relationship between exposure to contact allergens and induction of allergy (sensitisation) as well as elicitation is well established. This means that in principle, thresholds can be identified which are safe for the consumer.
- A model for dermal sensitisation quantitative risk assessment has been developed (QRA) and implemented by the fragrance industry. This model relies on thresholds, no effect or low-effect levels, established in healthy human volunteers and/or in animal experiments. The SCCP has previously reviewed this methodology and concluded that: “There is no confidence that the levels of skin sensitisers identified by the dermal sensitisation QRA are safe for the consumer.”
- Elicitation data can provide thresholds indicative for the safe use of those substances which have already caused significant problems in the consumer. In this context, “safe use” means that the thresholds will protect the majority of consumers from allergic contact dermatitis, but does not preclude that the most sensitive subset of the population may react upon exposure to the allergen.
- Furthermore, based on experience from intervention studies, such thresholds will also be sufficiently low to protect (most of) the non-sensitised consumers from developing contact allergy.
- Elicitation levels have been studied specifically for the fragrance chemicals cinnamal, hydroxycitronellal and isoeugenol. These studies, however, are not adequate to derive safe thresholds for the individual substances directly from the data.
- In the absence of adequate substance specific data it is possible to use a general threshold. Based on a statistical analysis of the available data in the scientific literature, a threshold of 0.8 µg/cm^2 was derived. This corresponds to 0.01% (100 ppm) limit in cosmetic products indicative for safe use.

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

- For substances for which there are no clinical data of concern, models such as the dermal sensitisation QRA approach may, after refinement and validation, be used to suggest a safe level of exposure prior to incorporation into products. However, aggregated exposures must be incorporated in the dermal sensitisation QRA model.

- HICC has for more than 10 years been recognized as an important allergen with more cases documented in the scientific literature than for any other fragrance chemical in this period. HICC has been shown to be a significant cause of disease as many of those with contact allergy to HICC had also reactions to cosmetics, which contained or were likely to contain HICC. Since 2003 attempts have been made by the fragrance industry to contain the outbreak of HICC allergy, but with no convincing success so far. Recent voluntary restrictions (recommendations to lower use concentrations, at least for some product types, to the level recommended by the SCCS in 2003) are not reflected in available evidence and are considered insufficient. The SCCS considers that the number of cases of HICC allergy documented over the last decade is exceptionally high and that continued exposure to HICC by the consumer is not considered safe, even at concentrations as low as 200 ppm. Therefore, HICC should not be used in consumer products in order to prevent further cases of contact allergy to HICC and to limit the consequences to those who already have become sensitized.

- The SCCP concluded in 2004 that chloroatranol and atranol, the main allergenic constituents of *Evernia prunastri and Evernia furfuracea*, should not be present in consumer products because they are extremly potent allergens. The persistently high frequency of contact allergy to *Evernia prunastri* and *Evernia furfuracea* noted in eczema patients does point to a persisting problem with exposure to the allergenic constituents, despite efforts to reduce the allergen content (296).

12. Data gaps and research needed

In the course of working on this opinion, the following points are highlighted as important data gaps, ordered by research area:

12.1. Clinical and epidemiological research

- Clinical data on more fragrance substances are needed to assess more fully the epidemiology of fragrance contact allergy and pin-point the culprit substances for induction and elicitation of contact allergy in man.
- Data from a broader range of EU countries on the clinical and epidemiological picture of fragrance contact allergy is needed, as difference in exposure and use habits are expected across Europe.
- A co-ordinated strategy for data collection should be developed.
- Very little is known about susceptible groups of the population, e.g. up 10% of the European population carry mutations, which impairs the skin barrier and which seem to increase the risk of fragrance allergy. Data are needed to qualify and quantify the increase in risk of susceptible groups in order to provide a better protection of all consumers.
- Aberrant enzyme activity in certain individuals, often related to genetic enzyme polymorphisms, may give an increased or reduced risk of sensitisation to prohaptens (that need enzymatic activation) in certain individuals or populations. More research into the role of relevant traits is needed.
- Dose-response data from clinical studies are available for only a few allergens. To establish individual safe levels such data are required for all established allergens of concern and covering an appropriate range of product types. This would also consolidate the basis of the use of a general threshold for safe use of fragrance allergens.
- Data on human exposure to fragrances from the use of different product categories is very scarce and therefore does not provide an optimal basis of risk assessment, e.g. exposure data on use for perfume/eau de cologne are lacking.
- Most experimental studies are done on individual fragrance ingredients, while exposure to allergens in cosmetic products is usually to mixtures of allergens. The risk of sensitisation and elicitation may depend on the mixture of substances, but very few studies on this exist. It is necessary to improve the knowledge base on cocktail effects on sensitisation/elicitation to improve the basis of risk assessment and management.
- Screening in dermatitis patients should be performed with air exposed samples of such fragrance substances that in experimental studies have been demonstrated to act as prehaptens, i.e. autoxidise and form oxidation mixtures containing allergenic oxidation products.
- Patch testing should if possible, be performed with the isolated true haptens formed from prehaptens and prohaptens to increase the possibility to diagnose allergy from these type of substances.
- There is a need for more experimental research to further establish the impact of the behaviour of fragrance substances when applied on the skin (including factors such as volatility, autoxidation, skin penetration, reactivity in skin and bioactivation).

12.2. Non-human studies

- Several studies in the industry submission (164) were of insufficient quality, not following the OECD guidelines.
- In some cases it was found that either very few concentrations points had been used in LLNAs, or concentrations were insufficient for achieving a 3-fold increase of the SI.

  A sufficient number of doses (concentrations) should be applied in LLNAs (at least 5) so that interpolation (for deriving an EC3 value) can rely on more than two or three actual data points to be more reliable. SCCS therefore suggests a change in the OECD guideline 429. (It is important to remember that the production of unreliable data is a waste of animals.) Moreover, the maximum concentration should be high enough to achieve a > 3-fold increase in SI, as far as this is possible with the substance/vehicle combination chosen.
- Data on experimental results are often not published, but available only on file in the companies having performed the tests. Access to such results would be important for the scientific community, e.g. in the context of REACH, or independently, either to the public domain, or to a Public Trustee.
- The OECD guideline 429 recommends several vehicles. It is well known that a difference in the EC3 value can be obtained for the same substance depending on which vehicle is used in the LLNA. Thus, as an additional control, supplementary to the guideline based LLNA control, a clinically relevant solvent or the commercial formulation in which the test substance is marketed may be used.
- As long as no validated *in vitro* method exists, more research is needed. Until one or more method(s) have been decided to fulfil the requirements for substituting *in vivo* testing, the *in vivo* testing for prediction of skin sensitisation has to be used.
- Applying only mechanism-based QSAR (QMM) as a tool in non-animal based risk assessment for skin sensitisation is of limited value for fragrance substances. This is due to major information gaps in the present model when addressing substances that act via abiotic or metabolic activation, and the high incidence of such substances in fragrances. Therefore, further experimental and clinical research in the area of abiotic and/or metabolic activation of fragrance substances is needed to increase the safety for the consumer, i.e. experimental studies which include air oxidation and bioactivation.
- Further experimental investigations of the sensitisation potential of fragrance substances are needed to determine the impact of the volatility of the substance as well as the effect of the vehicle on skin penetration/absorption and reactivity.
- From a clinical perspective it is important for the individual who is sensitised to one fragrance substance to know if they must also avoid other fragrance substances that can cause allergic contact dermatitis due to cross-reactivity with the original sensitiser. Prediction of risks for cross-reactivity requires sound application of theoretical principles in combination with well-designed experimental studies. This is a field that has not been studied very much so far and needs to be focused on much more in the future.
- Quantitative structure activity relationship (QSAR) models should be further developed, combining, as appropriate, information from *in silico*, *in chemico* and *in vitro* methods as possible. Prediction of different activation pathways should be included.
- Effect estimates such as proportions of sensitised humans or animals, or mean stimulation indices, EC3 values and other derivations should ideally be accompanied by an interval estimate (confidence interval) to address precision (297).

13. Opinion

Contact allergy to fragrances is a common, significant and relevant problem in Europe. The studies since the SCCNFP opinion on fragrance allergy in consumers in 1999 (SCCNFP/0017/98) (SCCNFP 1999) have confirmed that the 26 fragrance allergens, identified by the SCCNFP, are still relevant fragrance allergens for consumers because of their exposure from cosmetic products. Additional exposure to many of these 26 fragrance allergens also occurs from the use of other consumer products, such as detergents, toys, etc. Some of these fragrance substances are also used as preservatives.

The overall trend of fragrance contact allergy appears to have been stable for the last 10 years, as some causes of fragrance allergy have decreased and others increased. From the few population-based studies, it can be estimated that the frequency of contact allergy to fragrance ingredients in the general population in Europe is 1-3%. This is based on the limited testing with eight common fragrance allergens (FM I) out of the approximately 2500 fragrance ingredients listed in CosIng and indicative of the substances that may be present in fragrance compounds. However, the real prevalence of contact allergy to fragrance substances may be higher if the testing were to be performed with the full spectrum of fragrance allergens, including oxidised substances, where relevant.

Among eczema patients in the European population, around 16% are sensitised to fragrance ingredients. The disease can be severe and generalised, with a significant impairment of quality of life and potential consequences for fitness for work.

Contact sensitisation, and its clinical manifestation, allergic contact dermatitis, can be prevented if the exposure to known contact allergens is reduced or abolished (primary prevention). Experiences so far, have indicated that not all substances that later turned out to be significant contact allergens after human exposure, were predicted by experimental studies, e.g. the preservative methyldibromo glutaronitrile and the fragrance chemical HICC. Thus, a significant exposure of the population may occur before a substance is established as an important contact allergen in man.

Elicitation of allergic contact dermatitis occurs when a consumer sensitised to a certain substance is re-exposed to the substance in question. Prevention at this stage, termed secondary prevention, can be achieved if use of the allergen in products is eliminated or reduced to a tolerable level (general prevention), or if the patients succeed in avoiding all sources of exposure (individual prevention). Ingredient listing of individual fragrance allergens has been shown to be an important tool to enable consumers with an identified allergy to reduce/avoid relevant exposures. Moreover, ingredient listing is also of great importance to ensure that an adequate diagnosis of fragrance contact allergy can be made without undue delay. If the information given on the presence of fragrance allergens is incomplete, diagnosis of fragrance contact allergy may be missed.

The SCCNFP, in its 1999 opinion, identified 26 fragrance allergens for which information should be provided to consumers concerning their presence in cosmetic products. This was implemented in the European Cosmetics legislation (298) as ingredient labelling of these 26 fragrance substances (Annex III, entries 67-92). However, safe use concentrations for these substances in cosmetic products have not yet been determined and much new evidence concerning fragrance allergy has been published since 1999. The present opinion updates the SCCNFP opinion with a systematic and critical review of the scientific literature up to October 2010. This review addresses the issue of contact allergy to fragrance substances, including natural extracts and updates the list of fragrance allergens relevant to consumers. Clinical, epidemiological and experimental studies were evaluated, as well as modelling studies performed, to establish lists of: (i) established fragrance allergens; (ii) likely fragrance allergens; and (iii) possible fragrance allergens. The review also includes fragrances, which on modification by oxidation or by enzyme mediated processes, can produce allergens. Available dose-response data have been

examined to answer whether safe thresholds can be established for the most frequent fragrance allergens.

13.1. Question 1

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

In order to answer this question, the SCCS has used clinical and epidemiological data to identify known fragrance allergens. These were categorised as *established contact allergens in humans* (see Table 13-1).

Where sufficient animal evidence was present, these substances were categorised as established contact allergens in animals (Table 13-2). For a number of other fragrance substances, combinations of limited clinical data together with SAR considerations have been applied to indicate likely fragrance allergens in man (Table 13-3). Finally, SAR has also been applied to substances that lack human data to identify fragrance chemicals that have the structural potential to be contact allergens. Substances with insufficient human data were also considered as possible fragrance allergens. For these further tests (experimental/clinical data) are required (Table 13-4).

Table 13-1: Established contact allergens in humans.

For categorisation of importance (+ to ++++) see chapter 7.1. Allergens of special concern are substances where between 100 and 1,000 cases (+++) and more than 1,000 (++++) have been published. These are set in bold. Fragrance substances identified as allergens in the 1999 opinion of SCCNFP (1) are marked with an asterisk.
“ox.” = oxidised; “non-ox.” = non-oxidised; “r.t.” = rarely tested (see chapter 7)

INCI name (or, if none exists, perfuming name according to CosIng)	**CAS number**	**Human evidence: see text**
Individual chemicals		
ACETYLCEDRENE	32388-55-9	+
AMYL CINNAMAL*	122-40-7	++
AMYL CINNAMYL ALCOHOL*	101-85-9	++
AMYL SALICYLATE	2050-08-0	+
trans-ANETHOLE	4180-23-8	+ (r.t.)
ANISE ALCOHOL*	105-13-5	+
BENZALDEHYDE	100-52-7	+
BENZYL ALCOHOL*	100-51-6	++
BENZYL BENZOATE*	120-51-4	++
BENZYL CINNAMATE*	103-41-3	++
BENZYL SALICYLATE*	118-58-1	++
BUTYLPHENYL METHYLPROPIONAL *	80-54-6	++
CAMPHOR	76-22-2 / 464-49-3	+ (r.t.)
beta-CARYOPHYLLENE (ox.)	87-44-5	Non-ox.: +, ox.: +

INCI name (or, if none exists, perfuming name according to CosIng)	**CAS number**	**Human evidence: see text**
CARVONE	99-49-0 / 6485-40-1 / 2244-16-8	+ (r.t.)
CINNAMAL*	**104-55-2**	**+++**
CINNAMYL ALCOHOL*	**104-54-1**	**+++**
CITRAL*	**5392-40-5**	**+++**
CITRONELLOL*	106-22-9 / 1117-61-9 / 7540-51-4	++
COUMARIN*	**91-64-5**	**+++**
(DAMASCENONE) ROSE KETONE-4	23696-85-7	+ (r.t.)
alpha-DAMASCONE (TMCHB)	43052-87-5 / 23726-94-5	++
cis-beta-DAMASCONE	23726-92-3	+
delta-DAMASCONE	57378-68-4	+
DIMETHYLBENZYL CARBINYL ACETATE (DMBCA)	151-05-3	+
EUGENOL*	**97-53-0**	**+++**
FARNESOL*	**4602-84-0**	**++ - +++**
GERANIOL*	**106-24-1**	**+++**
HEXADECANOLACTONE	109-29-5	+ (r.t.)
HEXAMETHYLINDANOPYRAN	1222-05-5	++
HEXYL CINNAMAL*	101-86-0	++
HYDROXYISOHEXYL 3-CYCLOHEXENE CARBOXALDEHYDE *(HICC)**	**31906-04-4 / 51414-25-6**	**++++**
HYDROXYCITRONELLAL*	**107-75-5**	**+++**
ISOEUGENOL*	**97-54-1**	**+++**
alpha-ISOMETHYL IONONE*	127-51-5	++
(DL)-LIMONENE*	**138-86-3**	**++ (non-ox.);** **+++ (ox.)**
LINALOOL*	**78-70-6**	**++ (non-ox.)** **+++ (ox.)**
LINALYL ACETATE	115-95-7	+ (non-ox.) ++ (ox.)
MENTHOL	1490-04-6 / 89-78-1 / 2216-51-5	++
6-METHYL COUMARIN	92-48-8	++
METHYL 2-OCTYNOATE*	111-12-6	++
METHYL SALICYLATE	119-36-8	+
3-METHYL-5-(2,2,3-TRIMETHYL-3-	67801-20-1	++ (r.t.)

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

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

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

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

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

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

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

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

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

n.a.: not applicable (natural mixture)

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

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

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

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

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

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

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

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

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

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

*including their respective esters

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

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

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

Conclusions - Question 1

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

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

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

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

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

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

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

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

13.2. Question 2

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

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

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

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

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

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

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

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

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

Conclusions - Question 2

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

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

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

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

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

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

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

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

13.3. Question 3

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

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

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

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

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

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

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

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

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

Table 13-6: Known prehaptens and prohaptens.

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

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

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

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

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

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

Conclusions - Question 3

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

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

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

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

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

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

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

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

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

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

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

14. List of abbreviations

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

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

15. References

1. SCCNFP. The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers: Opinion concerning Fragrance Allergy in Consumers. A Review of the Problem. Analysis of the Need for appropriate Consumer Information and Identification of Consumer Allergens, adopted 8 December 1999. *SCCNFP/0017/98 Final* 1999:

2. Rustemeyer T, van Hoogstraten I M W, von Blomberg M E, Scheper R J. Mechanisms in Allergic Contact Dermatitis. In: Frosch P J, Menné T, Lepoittevin J P, eds. *Contact Dermatitis*. Heidelberg: Springer, 2006:

3. Johansen J D, Andersen T F, Kjoller M, Veien N, Avnstorp C, Andersen K E, Menne T. Identification of risk products for fragrance contact allergy: a case-referent study based on patients' histories. *Am J Contact Dermat* 1998: 9: 80-86.

4. Marks J G, Belsito D V, DeLeo V A, et al. North American Contact Dermatitis Group patch test results for the detection of delayed-type hypersensitivity to topical allergens. *J Am Acad Dermatol* 1998: 38: 911-918.

5. Johansen J D, Rastogi S C, Menné T. Contact allergy to popular perfumes: assessed by patch test, use test and chemical analysis. *Br J Dermatol* 1996: 135: 419-422.

6. Johansen J D, Rastogi S C, Andersen K E, Menne T. Content and reactivity to product perfumes in fragrance mix positive and negative eczema patients. A study of perfumes used in toiletries and skin-care products. *Contact Dermatitis* 1997: 36: 291-296.

7. de Groot A C, Frosch P J. Adverse reactions to fragrances. A clinical review. *Contact Dermatitis* 1997: 36: 57-86.

8. Cronin E. Contact Dermatitis. *Churchill Livingstone, Edinburgh* 1980:

9. Johansen J D, Andersen T F, Veien N, Avnstorp C, Andersen K E, Menne T. Patch testing with markers of fragrance contact allergy. Do clinical tests correspond to patients' self-reported problems? *Acta Derm Venereol* 1997: 77: 149-153.

10. Johansen J D, Rastogi S C, Bruze M, Andersen K E, Frosch P, Dreier B, Lepoittevin J P, White I, Menne T. Deodorants: a clinical provocation study in fragrance-sensitive individuals. *Contact Dermatitis* 1998: 39: 161-165.

11. Lammintausta K, Kalimo K, Havu V K. Occurrence of contact allergy and hand eczemas in hospital wet work. *Contact Dermatitis* 1982: 8: 84-90.

12. Meding B. Epidemiology of Hand Eczema in an Industrial City. *Acta Dermatol Venerol (Stockh) Suppl* 1990: 153: 2-43.

13. Heydorn S, Johansen J D, Andersen K E, Bruze M, Svedman C, White I R, Basketter D A, Menne T. Fragrance allergy in patients with hand eczema - a clinical study. *Contact Dermatitis* 2003: 48: 317-323.

14. Buckley D A, Rycroft R J, White I R, McFadden J P. Contact allergy to individual fragrance mix constituents in relation to primary site of dermatitis. *Contact Dermatitis* 2000: 43: 304-305.

15. Heydorn S, Menne T, Johansen J D. Fragrance allergy and hand eczema - a review. *Contact Dermatitis* 2003: 48: 59-66.

16. Wöhrl S, Hemmer W, Focke M, Götz M, Jarisch R. The significance of fragrance mix, balsam of Peru, colophony and propolis as screening tools in the detection of fragrance allergy. *Br J Dermatol* 2001: 145: 268-273.

17. Edman B. The influence of shaving method on perfume allergy. *Contact Dermatitis* 1994: 31: 291-292.

18. Heydorn S, Menne T, Andersen K E, Bruze M, Svedman C, White I R, Basketter D A. Citral a fragrance allergen and irritant. *Contact Dermatitis* 2003: 49: 32-36.

19. Rothenborg H W, Menne T, Sjolin K E. Temperature dependent primary irritant dermatitis from lemon perfume. *Contact Dermatitis* 1977: 3: 37-48.

20. Tanaka S, Matsumoto Y, Dlova N, Ostlere L S, Goldsmith P C, Rycroft R J, Basketter D A, White I R, Banerjee P, McFadden J P. Immediate contact reactions to fragrance mix constituents and Myroxylon pereirae resin. *Contact Dermatitis* 2004: 51: 20-21.

21. Hausen B M. Contact allergy to balsam of Peru. II. Patch test results in 102 patients with selected balsam of Peru constituents. *Am J Contact Dermat* 2001: 12: 93-102.

22. Katsarou A, Armenaka M, Ale I, Koufou V, Kalogeromitros D. Frequency of immediate reactions to the European standard series. *Contact Dermatitis* 1999: 41: 276-279.

23. Nakayama H. Perfume allergy and cosmetic dermatitis (in Japanese). *Jpn J Dermatol* 1974: 84: 659–667.

24. Nakayama H, Harada R, Toda M. Pigmented cosmetic dermatitis. *Int J Dermatol* 1981: 15: 673–675.

25. Cronin E. Photosensitivity to musk ambrette. *Contact Dermatitis* 1984: 11: 88-92.

26. Darvay A, White I R, Rycroft R J, Jones A B, Hawk J L, McFadden J P. Photoallergic contact dermatitis is uncommon. *Br J Dermatol* 2001: 145: 597-601.

27. Wang L, Sterling B, Don P. Berloque dermatitis induced by "Florida water". *Cutis* 2002: 70: 29-30.

28. Elberling J, Linneberg A, Dirksen A, Johansen J D, Frolund L, Madsen F, Nielsen N H, Mosbech H. Mucosal symptoms elicited by fragrance products in a population-based sample in relation to atopy and bronchial hyper-reactivity. *Clin Exp Allergy* 2005: 35: 75-81.

29. Kumar P, Caradonna-Graham V M, Gupta S, Cai X, Rao P N, Thompson J. Inhalation challenge effects of perfume scent strips in patients with asthma. *Ann Allergy Asthma Immunol* 1995: 75: 429-433.

30. Millqvist E, Bende M, Lowhagen O. Sensory hyperreactivity--a possible mechanism underlying cough and asthma-like symptoms. *Allergy* 1998: 53: 1208-1212.

31. Elberling J, Linneberg A, Mosbech H, Dirksen A, Frolund L, Madsen F, Nielsen N H, Johansen J D. A link between skin and airways regarding sensitivity to fragrance products? *Br J Dermatol* 2004: 151: 1197-1203.

32. Lindberg M, Matura M. Patch Testing. In: Johansen J D, Frosch P, Lepoittevin J P, eds. *Contact Dermatitis*. Heidelberg etc., : Springer, 2011: 439-464.

33. Basketter D. Diagnostic patch testing - does it have a wider relevance? *Contact Dermatitis* 2012: 67: 1-2.

34. Larsen W G. Perfume Dermatitis. A Study of 20 Patients. *Arch Dermatol* 1977: 113: 623-626.

35. Frosch P J, Pirker C, Rastogi S C, Andersen K E, Bruze M, Svedman C, Goossens A, White I R, Uter W, Arnau E G, Lepoittevin J P, Menne T, Johansen J D. Patch testing with a new fragrance mix detects additional patients sensitive to perfumes and missed by the current fragrance mix. *Contact Dermatitis* 2005: 52: 207-215.

36. Frosch P J, Rastogi S C, Pirker C, Brinkmeier T, Andersen K E, Bruze M, Svedman C, Goossens A, White I R, Uter W, Arnau E G, Lepoittevin J P, Johansen J D, Menne T. Patch testing with a new fragrance mix - reactivity to the individual constituents and chemical detection in relevant cosmetic products. *Contact Dermatitis* 2005: 52: 216-225.

37. Bruze M, Andersen K E, Goossens A. Recommendation to include fragrance mix 2 and hydroxyisohexyl 3-cyclohexene carboxaldehyde (Lyral) in the European baseline patch test series. *Contact Dermatitis* 2008: 58: 129-133.

38. Lindberg M, Edman B, Fischer T, Stenberg B. Time trends in Swedish patch test data from 1992 to 2000. A multi-centre study based on age- and sex-adjusted results of the Swedish standard series. *Contact Dermatitis* 2007: 56: 205-210.

39. Temesvari E, Nemeth I, Balo-Banga M J, Husz S, Kohanka V, Somos Z, Judak R, Remenyik E V, Szegedi A, Nebenfuhrer L, Meszaros C, Horvath A. Multicentre study of fragrance allergy in Hungary. Immediate and late type reactions. *Contact Dermatitis* 2002: 46: 325-330.

40. Machovcova A, Dastychova E, Kostalova D, Vojtechovska A, Reslova J, Smejkalova D, Vaneckova J, Vocilkova A. Common contact sensitizers in the Czech Republic. Patch test results in 12,058 patients with suspected contact dermatitis*. *Contact Dermatitis* 2005: 53: 162-166.

41. Lunder T, Kansky A. Increase in contact allergy to fragrances: patch-test results 1989-1998. *Contact Dermatitis* 2000: 43: 107-109.

42. Schnuch A, Lessmann H, Geier J, Frosch P J, Uter W. Contact allergy to fragrances: frequencies of sensitization from 1996 to 2002. Results of the IVDK*. *Contact Dermatitis* 2004: 50: 65-76.

43. Uter W, Geier J, Frosch P J, Schnuch A. Contact allergy to fragrances: current patch test results (2005 to 2008) from the IVDK network. *Contact Dermatitis* 2010: 63: 254-261.

44. van Oosten E J, Schuttelaar M L, Coenraads P J. Clinical relevance of positive patch test reactions to the 26 EU-labelled fragrances. *Contact Dermatitis* 2009: 61: 217-223.

45. deGroot A C, Coenraads P J, Bruynzeel D P, Jagtman B A, van_Ginkel C J W, Noz K, van_der_Valk P G M, Pavel S, Vink J, Weyland J W. Routine patch testing with fragrance chemicals in The Netherlands. *Contact Dermatitis* 2000: 42: 184-185.

46. Hendriks S A, van Ginkel C J. Evaluation of the fragrance mix in the European standard series. *Contact Dermatitis* 1999: 41: 161-162.

47. Nardelli A, Carbonez A, Ottoy W, Drieghe J, Goossens A. Frequency of and trends in fragrance allergy over a 15-year period. *Contact Dermatitis* 2008: 58: 134-141.

48. Brites M M, Goncalo M, Figueiredo A. Contact allergy to fragrance mix--a 10-year study. *Contact Dermatitis* 2000: 43: 181-182.

49. Cuesta L, Silvestre J F, Toledo F, Lucas A, Perez-Crespo M, Ballester I. Fragrance contact allergy: a 4-year retrospective study. *Contact Dermatitis* 2010: 63: 77-84.

50. Katsarma G, Gawkrodger D J. Suspected fragrance allergy requires extended patch testing to individual fragrance allergens. *Contact Dermatitis* 1999: 41: 193-197.

51. Buckley D A, Basketter D A, Kan-King-Yu D, White I R, White J L, McFadden J P. Atopy and contact allergy to fragrance: allergic reactions to the fragrance mix I (the Larsen mix). *Contact Dermatitis* 2008: 59: 220-225.

52. Thyssen J P, Carlsen B C, Menne T, Johansen J D. Trends of contact allergy to fragrance mix I and Myroxylon pereirae among Danish eczema patients tested between 1985 and 2007. *Contact Dermatitis* 2008: 59: 238-244.

53. Uter W, Hegewald J, Aberer W, Ayala F, Bircher A J, Brasch J, Coenraads P J, Schuttelaar M L, Elsner P, Fartasch M, Mahler V, Belloni Fortina A, Frosch P J, Fuchs T, Johansen J D, Menne T, Jolanki R, Krecisz B, Kiec-Swierczynska M, Larese F, Orton D, Peserico A, Rantanen T, Schnuch A. The European standard series in 9 European countries, 2002/2003 - First results of the European Surveillance System on Contact Allergies. *Contact Dermatitis* 2005: 53: 136-145.

54. Hegewald J, Uter W, Aberer W, Ayala F, Beliauskiene A, Belloni Fortina A, Bircher A, Brasch J, Chowdhury M M, Coenraads P J, Schuttelaer M-L, Elsner P, English J, Fartasch M, Mahler V, Frosch P J, Fuchs T, Gawkrodger D J, Giménez-Arnau A M, Green C M, Johansen J D, Menné T, Jolanki R, King C M, Krecisz B, Kiec-Swierczynska M, Larese F, Ormerod A D, Orton D, Peserico A, Rantanen T, Rustemeyer T, Sansom J E, Statham B N, Corradin M T, Wallnofer W, Wilkinson M, Schnuch A. The European Surveillance System of Contact Allergies (ESSCA): results of patch testing the standard series, 2004. *J Eur Acad Dermatol Venereol* 2008: 22: 174–181.

55. Uter W, Rämsch C, Aberer W, Ayala F, Balato A, Beliauskiene A, Fortina A B, Bircher A, Brasch J, Chowdhury M M, Coenraads P J, Schuttelaar M L, Cooper S, Corradin M T, Elsner P, English J S, Fartasch M, Mahler V, Frosch P J, Fuchs T, Gawkrodger D J, Gimenez-Arnau A M, Green C M, Horne H L, Jolanki R, King C M, Krecisz B, Kiec-Swierczynska M, Ormerod A D, Orton D I, Peserico A, Rantanen T, Rustemeyer T, Sansom J E, Simon D, Statham B N, Wilkinson M, Schnuch A. The European baseline series in 10 European Countries, 2005/2006--results of the European Surveillance System on Contact Allergies (ESSCA). *Contact Dermatitis* 2009: 61: 31-38.

56. An S, Lee A Y, Lee C H, Kim D W, Hahm J H, Kim K J, Moon K C, Won Y H, Ro Y S, Eun H C. Fragrance contact dermatitis in Korea: a joint study. *Contact Dermatitis* 2005: 53: 320-323.

57. Hussain I, Rani Z, Rashid T, Haroon T S. Suitability of the European standard series of patch test allergens in Pakistani patients. *Contact Dermatitis* 2002: 46: 50-51.

58. Gupta N, Shenoi S D, Balachandran C. Fragrance sensitivity in allergic contact dermatitis. *Contact Dermatitis* 1999: 40: 53-54.

59. Freireich-Astman M, David M, Trattner A. Standard patch test results in patients with contact dermatitis in Israel: age and sex differences. *Contact Dermatitis* 2007: 56: 103-107.

60. Lazarov A. European Standard Series patch test results from a contact dermatitis clinic in Israel during the 7-year period from 1998 to 2004. *Contact Dermatitis* 2006: 55: 73-76.

61. Kashani M N, Gorouhi F, Behnia F, Nazemi M J, Dowlati Y, Firooz A. Allergic contact dermatitis in Iran. *Contact Dermatitis* 2005: 52: 154-158.

62. Akyol A, Boyvat A, Peksari Y, Gurgey E. Contact sensitivity to standard series allergens in 1038 patients with contact dermatitis in Turkey. *Contact Dermatitis* 2005: 52: 333-337.

63. Lu X, Li L F, Wang W, Wang J. A clinical and patch test study of patients with positive patch test reactions to fragrance mix in China. *Contact Dermatitis* 2005: 52: 188-191.

64. Belsito D V, Fowler J F, Jr., Sasseville D, Marks J G, Jr., De Leo V A, Storrs F J. Delayed-type hypersensitivity to fragrance materials in a select North American population. *Dermatitis* 2006: 17: 23-28.

65. Zug K A, Warshaw E M, Fowler J F, Jr., Maibach H I, Belsito D L, Pratt M D, Sasseville D, Storrs F J, Taylor J S, Mathias C G, Deleo V A, Rietschel R L, Marks J. Patch-test results of the North American Contact Dermatitis Group 2005-2006. *Dermatitis* 2009: 20: 149-160.

66. Bruynzeel D P, Diepgen T L, Andersen K E, Brandao F M, Bruze M, Frosch P J, Goossens A, Lahti A, Mahler V, Maibach H I, Menne T, Wilkinson J D. Monitoring the European standard series in 10 centres 1996-2000. *Contact Dermatitis* 2005: 53: 146-149.

67. Frosch P J, Johansen J D, Menne T, Pirker C, Rastogi S C, Andersen K E, Bruze M, Goossens A, Lepoittevin J P, White I R. Further important sensitizers in patients sensitive to fragrances. I. Reactivity to 14 frequently used chemicals. *Contact Dermatitis* 2002: 47: 78-85.

68. Krautheim A, Uter W, Frosch P, Schnuch A, Geier J. Patch testing with fragrance mix II: results of the IVDK 2005-2008. *Contact Dermatitis* 2010: 63: 262-269.

69. Heisterberg M V, Andersen K E, Avnstorp C, al. e. Fragrance mix II in the baseline series contributes significantly to detection of fragrance allergy. *Contact Dermatitis* 2010: (accepted):

70. Frosch P J, Pilz B, Andersen K E, Burrows D, Camarasa J G, et al. Patch testing with fragrances: results of a multicenter study of the European Environmental and Contact Dermatitis Research Group with 48 frequently used constituents of perfumes. *Contact Dermatitis* 1995: 33: 333-342.

71. Frosch P J, Johansen J D, Menne T, Rastogi S C, Bruze M, Andersen K E, Lepoittevin J P, Gimenez Arnau E, Pirker C, Goossens A, White I R. Lyral is an important sensitizer in patients sensitive to fragrances. *Br J Dermatol* 1999: 141: 1076-1083.

72. Beliauskiene A, Valiukeviciene S, Uter W, Schnuch A. The European baseline series in Lithuania: results of patch testing in consecutive adult patients. *Journal of the European Academy of Dermatology and Venereology : JEADV* 2011: 25: 59-63.

73. Geier J, Brasch J, Schnuch A, Lessmann H, Pirker C, Frosch P J. Lyral has been included in the patch test standard series in Germany. *Contact Dermatitis* 2002: 46: 295-297.

74. Schnuch A, Uter W, Geier J, Lessmann H, Frosch P J. Sensitization to 26 fragrances to be labelled according to current European regulation. Results of the IVDK and review of the literature. *Contact Dermatitis* 2007: 57: 1-10.

75. Api A M. Only Peru Balsam extracts or distillates are used in perfumery. *Contact Dermatitis* 2006: 54: 179.

76. Avalos-Peralta P, Garcia-Bravo B, Camacho F M. Sensitivity to Myroxylon pereirae resin (balsam of Peru). A study of 50 cases. *Contact Dermatitis* 2005: 52: 304-306.

77. Cachao P, Menezes Brandao F, Carmo M, Frazao S, Silva M. Allergy to oil of turpentine in Portugal. *Contact Dermatitis* 1986: 14: 205-208.

78. Lear J T, Heagerty A H M, Tan B B, et al. Transient re-emergence of oil of turpentine allergy in the pottery industry. *Contact Dermatitis* 1996: 34: 169-172.

79. Treudler R, Richter G, Geier J, Schnuch A, Orfanos C E, Tebbe B. Increase in sensitization to oil of turpentine: recent data from a multicenter study on 45,005 patients from the German-Austrian Information Network of Departments of Dermatology (IVDK). *Contact Dermatitis* 2000: 42: 68-73.

80. Schäfer T, Böhler E, Ruhdorfer S, Weigl L, Wessner D, Filipiak B, Wichmann H E, Ring J. Epidemiology of contact allergy in adults. *Allergy* 2001: 56: 1192-1196.

81. Meding B, Swanbeck G. Occupational hand eczema in an industrial city. *Contact Dermatitis* 1990: 22: 13-23.

82. Nielsen N H, Menné T. Allergic contact sensitization in an unselected Danish population - the Glostrup allergy study, Denmark. *Acta Dermatol Venerol (Stockh)* 1992: 72: 456-460.

83. Meneghini C L, Sertoli A, Nava C, Angelini G, Francalani S, Foti C, Moroni P. Irritant contact dermatitis of the hands in housewives. In: Elsner P, Maibach H I, eds. *Irritant Dermatitis New Clinical and Experimental Aspects Curr Probl Dermatol*. Basel: Karger, 1995: 41-48.

84. Seidenari S, Manzini B M, Danese P, Motolese A. Patch and prick test study of 593 healthy subjects. *Contact Dermatitis* 1990: 23: 162-167.

85. Mørtz C G, Bindslev-Jensen C, Lauritsen J, Andersen K E. Allergic contact sensitization in 8th grade school children in Odense, Denmark. . *Abstract presented at the Jadassohn Centenary Congress, London 9-12 Oct 1996* 1996:

86. Guin J D, Berry V K. Perfume sensitivity in adult females. A study of contact sensitivity to a perfume mix in two groups of student nurses. *J Am Acad Dermatol* 1980: 3: 299-302.

87. Nielsen N H, Linneberg A, Menne T, Madsen F, Frolund L, Dirksen A, Jorgensen T. Allergic contact sensitization in an adult Danish population: two cross-sectional surveys eight years apart (the Copenhagen Allergy Study). *Acta Derm Venereol* 2001: 81: 31-34.

88. Thyssen J P, Linneberg A, Menne T, Nielsen N H, Johansen J D. The prevalence and morbidity of sensitization to fragrance mix I in the general population. *Br J Dermatol* 2009: 161: 95-101.

89. Buckley D A, Rycroft R J, White I R, McFadden J P. The frequency of fragrance allergy in patch-tested patients increases with their age. *Br J Dermatol* 2003: 149: 986-989.

90. Uter W, Schnuch A, Geier J, Pfahlberg A, Gefeller O. Association between occupation and contact allergy to the fragrance mix: a multifactorial analysis of national surveillance data. *Occup Environ Med* 2001: 58: 392-398.

91. Dotterud L K, Smith-Sivertsen T. Allergic contact sensitization in the general adult population: a population-based study from Northern Norway. *Contact Dermatitis* 2007: 56: 10-15.

92. Smith-Sivertsen T, Dotterud L K, Lund E. Nickel allergy and its relationship with local nickel pollution, ear piercing, and atopic dermatitis: a population-based study from Norway. *J Am Acad Dermatol* 1999: 40: 726-735.

93. White J M, Gilmour N J, Jeffries D, Duangdeeden I, Kullavanijaya P, Basketter D A, McFadden J P. A general population from Thailand: incidence of common allergens with emphasis on para-phenylenediamine. *Clin Exp Allergy* 2007: 37: 1848-1853.

94. Bruze M. What is a relevant contact allergy? *Contact Dermatitis* 1990: 23: 224-225.

95. Ale I, Maibach H I. Clinical Relevance in Allergic Contact Dermatitis. An algorithmic approach. *Derm Beruf Umwelt* 1995: 43: 119-121.

96. Wahlberg J E, Lindberg M. Patch Testing. In: Frosch P J, Menné T, Lepoittevin J P, eds. *Contact Dermatitis*. Berlin: Springer, 2006: 365-390.

97. Frosch P J, Johansen J D, Menne T, Pirker C, Rastogi S C, Andersen K E, Bruze M, Goossens A, Lepoittevin J P, White I R. Further important sensitizers in patients sensitive to fragrances. II. Reactivity to essential oils. *Contact Dermatitis* 2002: 47: 279-287.

98. Rothenborg H W, Hjorth N. Allergy to perfumes from toilet soaps and detergents in patients with dermatitis. *Arch Dermatol* 1968: 97: 417-421.

99. Hannuksela M, Kousa M, Pirila V. Allergy to ingredients of vehicles. *Contact Dermatitis* 1976: 2: 105-110.

100. Johansen J D, Andersen K E, Menné T. Quantitative aspects of isoeugenol contact allergy assessed by use and patch tests. *Contact Dermatitis* 1996: 34: 414-418.

101. Johansen J D, Andersen K E, Rastogi S C, Menne T. Threshold responses in cinnamic-aldehyde-sensitive subjects: results and methodological aspects. *Contact Dermatitis* 1996: 34: 165-171.

102. Jorgensen P H, Jensen C D, Rastogi S, Andersen K E, Johansen J D. Experimental elicitation with hydroxyisohexyl-3-cyclohexene carboxaldehyde-containing deodorants. *Contact Dermatitis* 2007: 56: 146-150.

103. Bruze M, Johansen J D, Andersen K E, Frosch P, Lepoittevin J P, Rastogi S, Wakelin S, White I, Menne T. Deodorants: an experimental provocation study with cinnamic aldehyde. *J Am Acad Dermatol* 2003: 48: 194-200.

104. Svedman C, Bruze M, Johansen J D, Andersen K E, Goossens A, Frosch P J, Lepoittevin J P, Rastogi S, White I R, Menne T. Deodorants: an experimental provocation study with hydroxycitronellal. *Contact Dermatitis* 2003: 48: 217-223.

105. Schnuch A, Uter W, Dickel H, Szliska C, Schliemann S, Eben R, Rueff F, Gimenez-Arnau A, Loffler H, Aberer W, Frambach Y, Worm M, Niebuhr M, Hillen U, Martin V, Jappe U, Frosch P J, Mahler V. Quantitative patch and repeated open application testing in hydroxyisohexyl 3-cyclohexene carboxaldehyde sensitive-patients. *Contact Dermatitis* 2009: 61: 152-162.

106. Uter W, Geier J, Schnuch A, Frosch P J. Patch test results with patients' own perfumes, deodorants and shaving lotions: results of the IVDK 1998-2002. *J Eur Acad Dermatol Venereol* 2007: 21: 374-379.

107. Basra M K, Fenech R, Gatt R M, Salek M S, Finlay A Y. The Dermatology Life Quality Index 1994-2007: a comprehensive review of validation data and clinical results. *Br J Dermatol* 2008: 159: 997-1035.

108. Skoet R, Zachariae R, Agner T. Contact dermatitis and quality of life: a structured review of the literature. *Br J Dermatol* 2003: 149: 452-456.

109. Moberg C, Alderling M, Meding B. Hand eczema and quality of life: a population-based study. *Br J Dermatol* 2009: 161: 397-403.

110. Meding B, Swanbeck G. Consequences of having hand eczema. *Contact Dermatitis* 1990: 23: 6-14.

111. Agner T, Andersen K E, Brandao F M, Bruynzeel D P, Bruze M, Frosch P, Goncalo M, Goossens A, Le Coz C J, Rustemeyer T, White I R, Diepgen T. Contact sensitisation in hand eczema patients-relation to subdiagnosis, severity and quality of life: a multi-centre study. *Contact Dermatitis* 2009: 61: 291-296.

112. Lysdal S H, Johansen J D. Fragrance contact allergic patients: strategies for use of cosmetic products and perceived impact on life situation. *Contact Dermatitis* 2009: 61: 320-324.

113. Meding B, Wrangsjo K, Jarvholm B. Fifteen-year follow-up of hand eczema: predictive factors. *J Invest Dermatol* 2005: 124: 893-897.

114. Hald M, Agner T, Blands J, Ravn H, Johansen J D. Allergens associated with severe symptoms of hand eczema and a poor prognosis. *Contact Dermatitis* 2009: 61: 101-108.

115. Wijnhoven S W P, Ezendam J, Schuur A G, van Loveren H, van Engelen J G M. *Allergens in consumer products. RIVM Reprot 320025001*. Bilthoven: Institute for Public Health and the Environment, 2008.

116. Schnuch A, Aberer W, Agathos M, Becker D, Brasch J, Elsner P, Frosch P J, Fuchs T, Geier J, Hillen U, Löffler H, Mahler V, Richter G, Szliska C. Durchführung des Epikutantests mit Kontaktallergenen. Leitlinien der Deutschen Dermatologischen Gesellschaft; Deutschen Gesellschaft für Allergie und klinische Immunologie. *J Dtsch Dermatol Ges* 2008: 6: 770-775.

117. Scheinmann P L. The foul side of fragrance-free products: What every clinician should know about managing patients with fragrance allergy. *J Am Acad Dermatol* 1999: 41: 1020-1024.

118. Hagvall L, Backtorp C, Norrby P O, Karlberg A T, Borje A. Experimental and theoretical investigations of the autoxidation of geranial: a dioxolane hydroperoxide identified as a skin sensitizer. *Chemical research in toxicology* 2011: 24: 1507-1515.

119. Hagvall L, Backtorp C, Svensson S, Nyman G, Borje A, Karlberg A T. Fragrance compound geraniol forms contact allergens on air exposure. Identification and quantification of oxidation products and effect on skin sensitization. *Chem Res Toxicol* 2007: 20: 807-814.

120. Hagvall L, Baron J M, Borje A, Weidolf L, Merk H, Karlberg A T. Cytochrome P450-mediated activation of the fragrance compound geraniol forms potent contact allergens. *Toxicol Appl Pharmacol* 2008: 233: 308-313.

121. Brared Christensson J, Matura M, Backtorp C, Borje A, Nilsson J L, Karlberg A T. Hydroperoxides form specific antigens in contact allergy. *Contact Dermatitis* 2006: 55: 230-237.

122. Karlberg A T, Bergstrom M A, Borje A, Luthman K, Nilsson J L. Allergic contact dermatitis--formation, structural requirements, and reactivity of skin sensitizers. *Chem Res Toxicol* 2008: 21: 53-69.

123. Karlberg A T, Boman A, Melin B. Animal experiments on the allergenicity of d-limonene--the citrus solvent. *Ann Occup Hyg* 1991: 35: 419-426.

124. Karlberg A T, Magnusson K, Nilsson U. Air oxidation of d-limonene (the citrus solvent) creates potent allergens. *Contact Dermatitis* 1992: 26: 332-340.

125. Karlberg A T, Shao L P, Nilsson U, Gafvert E, Nilsson J L. Hydroperoxides in oxidized d-limonene identified as potent contact allergens. *Arch Dermatol Res* 1994: 286: 97-103.

126. Sköld M, Börje A, Matura M, Karlberg A T. Studies on the autoxidation and sensitizing capacity of the fragrance chemical linalool, identifying a linalool hydroperoxide. *Contact Dermatitis* 2002: 46: 267-272.

127. Sköld M, Börje A, Harambasic E, Karlberg A T. Contact allergens formed on air exposure of linalool. Identification and quantification of primary and secondary oxidation products and the effect on skin sensitization. *Chem Res Toxicol* 2004: 17: 1697-1705.

128. Sköld M, Hagvall L, Karlberg A T. Autoxidation of linalyl acetate, the main component of lavender oil, creates potent contact allergens. *Contact Dermatitis* 2008: 58: 9-14.

129. Rudback J, Bergstrom M A, Borje A, Nilsson U, Karlberg A T. alpha-Terpinene, an antioxidant in tea tree oil, autoxidizes rapidly to skin allergens on air exposure. *Chem Res Toxicol* 2012: 25: 713-721.

130. Karlberg A T, Dooms-Gossens A. Contact allergy to oxidized d-limonene among dermatitis patients. *Contact Dermatitis* 1997: 36: 201-206.

131. Matura M, Goossens A, Bordalo O, Garcia-Bravo B, Magnusson K, Wrangsjo K, Karlberg A T. Oxidized citrus oil (R-limonene): a frequent skin sensitizer in Europe. *J Am Acad Dermatol* 2002: 47: 709-714.

132. Matura M, Goossens A, Bordalo O, Garcia-Bravo B, Magnusson K, Wrangsjo K, Karlberg A T. Patch testing with oxidized R-(+)-limonene and its hydroperoxide fraction. *Contact Dermatitis* 2003: 49: 15-21.

133. Matura M, Skold M, Borje A, Andersen K E, Bruze M, Frosch P, Goossens A, Johansen J D, Svedman C, White I R, Karlberg A T. Selected oxidized fragrance terpenes are common contact allergens. *Contact Dermatitis* 2005: 52: 320-328.

134. Matura M, Skold M, Borje A, Andersen K E, Bruze M, Frosch P, Goossens A, Johansen J D, Svedman C, White I R, Karlberg A T. Not only oxidized R-(+)- but also S-(-)-limonene is a common cause of contact allergy in dermatitis patients in Europe. *Contact Dermatitis* 2006: 55: 274-279.

135. Christensson J B, Matura M, Gruvberger B, Bruze M, Karlberg A T. Linalool--a significant contact sensitizer after air exposure. *Contact Dermatitis* 2010: 62: 32-41.

136. Sköld M, Karlberg A T, Matura M, Börje A. The fragrance chemical beta-caryophyllene-air oxidation and skin sensitization. *Food Chem Toxicol* 2006: 44: 538-545.

137. Santucci B, Cristaudo A, Cannistraci C, Picardo M. Contact dermatitis to fragrances. *Contact Dermatitis* 1987: 16: 93-95.

138. Fregert S, Hjorth N. Results of Standard Patch Tests with Substances Abandoned. *Contact Dermatitis Newsletter* 1969: 5: 85-86.

139. de Groot A C, Liem D H, Nater J P, van Ketel W G. Patch tests with fragrance materials and preservatives. *Contact Dermatitis* 1985: 12: 87-92.

140. Hagvall L, Skold M, Brared-Christensson J, Borje A, Karlberg A T. Lavender oil lacks natural protection against autoxidation, forming strong contact allergens on air exposure. *Contact Dermatitis* 2008: 59: 143-150.

141. Berglund V. *Master Thesis University of Gothenburg*. 2011.

142. Ruberto G, Baratta M T, Deans S G, Dorman H J. Antioxidant and antimicrobial activity of Foeniculum vulgare and Crithmum maritimum essential oils. *Planta Med* 2000: 66: 687-693.

143. Kim H J, Chen F, Wu C, Wang X, Chung H Y, Jin Z. Evaluation of antioxidant activity of Australian tea tree (Melaleuca alternifolia) oil and its components. *J Agric Food Chem* 2004: 52: 2849-2854.

144. Foti M C, Ingold K U. Mechanism of inhibition of lipid peroxidation by gamma-terpinene, an unusual and potentially useful hydrocarbon antioxidant. *J Agric Food Chem* 2003: 51: 2758-2765.

145. Buckley D A. Allergy to oxidized linalool in the UK. *Contact Dermatitis* 2011: 64: 240-241.

146. Smith C K, Hotchkiss S A. Enzymes and mechanisms of xenobiotic metabolism. In: editor?)) w i, eds. *Allergic Contact Dermatitis Chemical and Metabolic Mechanisms*. Taylor and Francis, London and New York, 2001: 45-87.

147. Kalgutkar A S, Gardner I, Obach R S, Shaffer C L, Callegari E, Henne K R, Mutlib A E, Dalvie D K, Lee J S, Nakai Y, O'Donnell J P, Boer J, Harriman S P. A comprehensive listing of bioactivation pathways of organic functional groups. *Curr Drug Metab* 2005: 6: 161-225.

148. Nilsson A M, Bergstrom M A, Luthman K, Nilsson J L, Karlberg A T. A conjugated diene identified as a prohapten: contact allergenic activity and chemical reactivity of proposed epoxide metabolites. *Chem Res Toxicol* 2005: 18: 308-316.

149. Bergström M A, Luthman K, Nilsson J L, Karlberg A T. Conjugated dienes as prohaptens in contact allergy: in vivo and in vitro studies of structure-activity relationships, sensitizing capacity, and metabolic activation. *Chem Res Toxicol* 2006: 19: 760-769.

150. Bergström M A, Ott H, Carlsson A, Neis M, Zwadlo-Klarwasser G, Jonsson C A, Merk H F, Karlberg A T, Baron J M. A skin-like cytochrome P450 cocktail activates prohaptens to contact allergenic metabolites. *J Invest Dermatol* 2007: 127: 1145-1153.

151. Basketter D A. Skin Sensitization to Cinnamic Alcohol: The Role of Skin Metabolism. *Acta Derm Venereol* 1992: 72: 264-265.

152. Smith C K, Moore C A, Elahi E N, Smart A T, Hotchkiss S A. Human skin absorption and metabolism of the contact allergens, cinnamic aldehyde, and cinnamic alcohol. *Toxicol Appl Pharmacol* 2000: 168: 189-199.

153. Cheung C, Hotchkiss S A, Pease C K. Cinnamic compound metabolism in human skin and the role metabolism may play in determining relative sensitisation potency. *J Dermatol Sci* 2003: 31: 9-19.

154. Elahi E N, Wright Z, Hinselwood D, Hotchkiss S A, Basketter D A, Pease C K. Protein binding and metabolism influence the relative skin sensitization potential of cinnamic compounds. *Chem Res Toxicol* 2004: 17: 301-310.

155. Ott H, Wiederholt T, Bergstrom M A, Heise R, Skazik C, Czaja K, Marquardt Y, Karlberg A T, Merk H F, Baron J M. High-resolution transcriptional profiling of chemical-stimulated dendritic cells identifies immunogenic contact allergens, but not prohaptens. *Skin Pharmacol Physiol* 2010: 23: 213-224.

156. Bertrand F, Basketter D A, Roberts D W, Lepoittevin J P. Skin sensitization to eugenol and isoeugenol in mice: possible metabolic pathways involving ortho-quinone and quinone methide intermediates. *Chemical research in toxicology* 1997: 10: 335-343.

157. Rastogi S C, Johansen J D. Significant exposures to isoeugenol derivatives in perfumes. *Contact Dermatitis* 2008: 58: 278-281.

158. Flyvholm M A, Andersen K E, Baranski B, Sarlo K. *Criteria for classification of skin- and airway-sensitizing substances in the work and general environments*. Regional Office for Europe: WHO, 1996.

159. Basketter D A, Flyvholm M A, Menne T. Classification criteria for skin-sensitizing chemicals: a commentary. *Contact Dermatitis* 1999: 40: 175-182.

160. Schnuch A, Lessmann H, Schulz K H, Becker D, Diepgen T L, Drexler H, Erdmann S, Fartasch M, Greim H, Kricke-Helling P, Merget R, Merk H, Nowak D, Rothe A, Stropp G, Uter W, Wallenstein G. When should a substance be designated as sensitizing for the skin ('Sh') or for the airways ('Sa')? *Hum Exp Toxicol* 2002: 21: 439-444.

161. Basketter D A, Andersen K E, Liden C, Van Loveren H, Boman A, Kimber I, Alanko K, Berggren E. Evaluation of the skin sensitizing potency of chemicals by using the existing methods and considerations of relevance for elicitation. *Contact Dermatitis* 2005: 52: 39-43.

162. Anonymous. *Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. ECHA Reference: ECHA-11-G-06-EN. Date: 04/2011* http://echa.europa.eu/documents/10162/17217/clp_en.pdf 2011.

163. Lalko J, Api A M, Politano V T, Letizia C. Quantitative risk assessment for dermal sensitization to fragrance ingrediets: The utility of LLNA data in the weight of evidence approach to identifying thresholds. *46th Congress of the European Societies of Toxicology, September 13-16 2009, Dresden, Germany* 2009:

164. RIFM. Local lymph node assay (LLNA) protocol summaries: Data presented at the 46th Congress of the European Societies of Toxicology. *Research Institute for Fragrance Materials, Inc* 2009:

165. Gerberick G F, Kern P S, Schlatter H, Dearman R J, Kimber I, Patlewicz G Y, Basketter D A. Compilation of historical local lymph node data for evaluation of skin sensitization alternative methods *Dermatitis* 2005: 16: 157-202.

166. Kern P S, Gerberick G F, Ryan C A, Kimber I, Aptula A, Basketter D A. Local lymph node data for the evaluation of skin sensitization alternatives: a second compilation. *Dermatitis* 2010: 21: 8-32.

167. Arnau E G, Andersen K E, Bruze M, Frosch P J, Johansen J D, Menne T, Rastogi S C, White I R, Lepoittevin J P. Identification of Lilial as a fragrance sensitizer in a perfume by bioassay-guided chemical fractionation and structure-activity relationships. *Contact Dermatitis* 2000: 43: 351-358.

168. Lepoittevin J P. Metabolism versus chemical transformation or pro- versus prehaptens? *Contact Dermatitis* 2006: 54: 73-74.

169. SCCP. Opinion concerning the predictive testing of potentially cutaneous sensiting cosmetic ingredients or mixtures of ingredients adopted by the SCCNFP during the 11th plenary session of 17 February 2000. 2000:

170. Heisterberg M V, Menne T, Johansen J D. Contact allergy to the 26 specific fragrance ingredients to be declared on cosmetic products in accordance with the EU cosmetics directive. *Contact Dermatitis* 2011: 65: 266-275.

171. Larsen W, Nakayama H, Fischer T, Elsner P, Frosch P, Burrows D, Jordan W, Shaw S, Wilkinson J, Marks J, Jr., Sugawara M, Nethercott M, Nethercott J. Fragrance contact dermatitis: a worldwide multicenter investigation (Part II). *Contact Dermatitis* 2001: 44: 344-346.

172. Andersen A. Final report on the safety assessment of sodium p-chloro-m-cresol, p-chloro-m-cresol, chlorothymol, mixed cresols, m-cresol, o-cresol, p-cresol, isopropyl cresols, thymol, o-cymen-5-ol, and carvacrol. *Int J Toxicol* 2006: 25 Suppl 1: 29-127.

173. Larsen W, Nakayama H, Fischer T, Elsner P, Frosch P, Burrows D, Jordan W, Shaw S, Wilkinson J, Marks J, Sugawara M, Nethercott M, Nethercott J. Fragrance contact dermatitis - a worldwide multicenter investigation (Part III). *Contact Dermatitis* 2002: 46: 141-144.

174. Lapczynski A, Lalko J, McGinty D, Bhatia S, Letizia C S, Api A M. Fragrance material review on trans,trans-delta-damascone. *Food Chem Toxicol* 2007: 45 Suppl 1: S211-215.

175. Letizia C S, Cocchiara J, Wellington G A, Funk C, Api A M. Food and chemical toxicology. *Food Chem Toxicol* 2000: 38 Suppl 3: S1-236.

176. Mitchell D M, Beck M H. Contact allergy to benzyl alcohol in a cutting oil reodorant. *Contact Dermatitis* 1988: 18: 301-302.

177. Mitchell J C. Contact hypersensitivity to some perfume materials. *Contact Dermatitis* 1975: 1: 196-199.

178. Malten K E, van Ketel W G, Nater J P, Liem D H. Reactions in selected patients to 22 fragrance materials. *Contact Dermatitis* 1984: 11: 1-10.

179. Mitchell J C, Calnan C D, Clendenning W E, Cronin E, Hjorth N, Magnusson B, Maibach H I, Meneghini C L, Wilkinson D S. Patch testing with some components of balsam of Peru. *Contact Dermatitis* 1976: 2: 57-58.

180. Bernaola G, Escayol P, Fernandez E, de Corres L F. Contact dermatitis from methylionone fragrance. *Contact Dermatitis* 1989: 20: 71-72.

181. English J S, Rycroft R J. Allergic contact dermatitis from methyl heptine and methyl octine carbonates. *Contact Dermatitis* 1988: 18: 174-175.

182. Bhatia S P, McGinty D, Letizia C S, Api A M. Fragrance material review on myrtenol. *Food Chem Toxicol* 2008: 46 Suppl 11: S237-240.

183. Lapczynski A, Bhatia S P, Letizia C S, Api A M. Fragrance material review on nerolidol (isomer unspecified). *Food Chem Toxicol* 2008: 46 Suppl 11: S247-250.

184. Sanchez-Politta S, Campanelli A, Pashe-Koo F, Saurat J H, Piletta P. Allergic contact dermatitis to phenylacetaldehyde: a forgotten allergen? *Contact Dermatitis* 2007: 56: 171-172.

185. McGinty D, Letizia C S, Api A M. Fragrance material review on phytol. *Food Chem Toxicol* 2010: 48 Suppl 3: S59-63.

186. Lapczynski A, Bhatia S P, Letizia C S, Api A M. Fragrance material review on rhodinol. *Food Chem Toxicol* 2008: 46 Suppl 11: S259-262.

187. Lapczynski A, Lalko J, McGinty D, Bhatia S P, Letizia C S, Api A M. Fragrance material review on alpha-isodamascone. *Food Chem Toxicol* 2007: 45 Suppl 1: S267-271.

188. Trattner A, David M. Patch testing with fine fragrances: comparison with fragrance mix, balsam of Peru and a fragrance series. *Contact Dermatitis* 2003: 49: 287-289.

189. Surburg H, Panten J. *Common fragrance and flavor materials: preparation, properties and uses*. Weinheim: Wiley-VCH, 2006.

190. Schmidt E. Production of Essential Oils. In: Husnu Can Baser K, Buchbauer G, eds. *Handbook of Essential Oils - Science, Technology, and Applications*. Boca Raton: CRC Press, 2010: 88-95.

191. Trattner A, David M, Lazarov A. Occupational contact dermatitis due to essential oils. *Contact Dermatitis* 2008: 58: 282-284.

192. Jung P, Sesztak-Greinecker G, Wantke F, Gotz M, Jarisch R, Hemmer W. Mechanical irritation triggering allergic contact dermatitis from essential oils in a masseur. *Contact Dermatitis* 2006: 54: 297-299.

193. Bilsland D, Strong A. Allergic contact dermatitis from the essential oil of French marigold (Tagetes patula) in an aromatherapist. *Contact Dermatitis* 1990: 23: 55-56.

194. Cockayne S E, Gawkrodger D J. Occupational contact dermatitis in an aromatherapist. *Contact Dermatitis* 1997: 37: 306-307.

195. Boonchai W, Iamtharachai P, Sunthonpalin P. Occupational allergic contact dermatitis from essential oils in aromatherapists. *Contact Dermatitis* 2007: 56: 181-182.

196. Keane F M, Smith H R, White I R, Rycroft R J. Occupational allergic contact dermatitis in two aromatherapists. *Contact Dermatitis* 2000: 43: 49-51.

197. Selvaag E, Holm J O, Thune P. Allergic contact dermatitis in an aroma therapist with multiple sensitizations to essential oils. *Contact Dermatitis* 1995: 33: 354-355.

198. Romaguera C, Vilaplana J. Occupational contact dermatitis from ylang-ylang oil. *Contact Dermatitis* 2000: 43: 251.

199. Rudzki E, Grzywa Z. Allergy to perfume mixture. *Contact Dermatitis* 1986: 15: 115-116.

200. Rudzki E, Grzywa Z, Bruo W S. Sensitivity to 35 essential oils. *Contact Dermatitis* 1976: 2: 196-200.

201. Vilaplana J, Romaguera C. Contact dermatitis from the essential oil of tangerine in fragrance. *Contact Dermatitis* 2002: 46: 108.

202. Rudzki E, Grzywa Z. Sensitizing and irritating properties of star anise oil. *Contact Dermatitis* 1976: 2: 305-308.

203. Sugiura M, Hayakawa R, Kato Y, Sugiura K, Hashimoto R. Results of patch testing with lavender oil in Japan. *Contact Dermatitis* 2000: 43: 157-160.

204. Lalko J, Api A M. Investigation of the dermal sensitization potential of various essential oils in the local lymph node assay. *Food Chem Toxicol* 2006: 44: 739-746.

205. SCCP. *Memorandum Classification and categorization of skin sensitisers and grading of test reactions (SCCP/0919/05). Scientific Committee for on Consumer Protection, adopted 20 September 2005*. 2005.

206. SCCP. *Memorandum on Hair Dye Substances and their Skin Sensitising Properties. Scientific Committee on Consumer Protection, adopted 19 December 2006*. 2006.

207. Christensson J B, Johansson S, Hagvall L, Jonsson C, Borje A, Karlberg A T. Limonene hydroperoxide analogues differ in allergenic activity. *Contact Dermatitis* 2008: 59: 344-352.

208. Landsteiner K, Jacobs J. Studies on the sensitization of animals with simple chemical compounds. *J Exp Med* 1936: 64: 625-629.

209. Ridriguez E, Towers G H N, Mitchell J C. Biological aspects of sequiterpene lactones. *Phytochemistry* 1976: 15: 1573-1580.

210. Roberts D W, Goodwin B F J, Williams D L, Jones K, Johnson A W, Alderson J C E. Correlation between skin sensitization potential and chemical reactivity for nitrobenzyl compounds. *Food Chem Toxicol* 1984: 21: 811-813.

211. Dupuis G, Benezra C. *Allergic contact dermatitis to simple chemicals: a molecular approach* New York: Marcel Dekker, 1982.

212. Smith C K, Hotchkiss S A. *Allergic Contact Dermatitis, Chemical and Metabolic Mechanisms*. London: Taylor and Francis, 2001.

213. Roberts D W, Lepoittevin J P. Hapten-Protein Interactions. In: Lepoittevin J P, Basketter D, Goossens A, Karlberg A T, eds. *Allergic Contact Dermatitis The Molecular Basis*. Heidelberg: Springer, 1998:

214. Sykes P. *A guidebook to mechanism in organic chemistry* Edinburgh: Pearson, 1961.

215. Aptula A O, Roberts D W. Mechanistic applicability domains for nonanimal-based prediction of toxicological end points: general principles and application to reactive toxicity. *Chem Res Toxicol* 2006: 19: 1097-1105.

216. Gerberick G F, Vassallo J D, Bailey R E, Chaney J G, Morrall S W, Lepoittevin J P. Development of a peptide reactivity assay for screening contact allergens. *Toxicological sciences : an official journal of the Society of Toxicology* 2004: 81: 332-343.

217. Natsch A, Gfeller H, Rothaupt M, Ellis G. Utility and limitations of a peptide reactivity assay to predict fragrance allergens in vitro. *Toxicology in vitro : an international journal published in association with BIBRA* 2007: 21: 1220-1226.

218. Gerberick G F, Troutman J A, Foertsch L M, Vassallo J D, Quijano M, Dobson R L, Goebel C, Lepoittevin J P. Investigation of peptide reactivity of pro-hapten skin sensitizers using a peroxidase-peroxide oxidation system. *Toxicological sciences : an official journal of the Society of Toxicology* 2009: 112: 164-174.

219. Troutman J A, Foertsch L M, Kern P S, Dai H J, Quijano M, Dobson R L M, Lalko J F, Lepoittevin J-P, Gerberick G F. The incorporation of lysine into the peroxidase peptide reactivity assay for skin sensitization assessments. *Toxicol Sci* 2011: 122: 422-436.

220. Roberts D W, Aptula A O, Patlewicz G. Mechanistic applicability domains for non-animal based prediction of toxicological endpoints. QSAR analysis of the schiff base applicability domain for skin sensitization. *Chem Res Toxicol* 2006: 19: 1228-1233.

221. Roberts D W, Aptula A O, Patlewicz G Y. Chemistry-Based Risk Assessment for Skin Sensitization: Quantitative Mechanistic Modeling for the S(N)Ar Domain. *Chem Res Toxicol* 2011:

222. Johansen J D. Contact allergy to fragrances: clinical and experimental investigations of the fragrance mix and its ingredients. *Contact Dermatitis* 2002: 46 (suppl. 3): 4-31.

223. Fenn R S. Aroma chemical usage trends in modern perfumery. *Perfumer Flavorist* 1989: 14: 1-10.

224. Johansen J D, Frosch P J, Svedman C, Andersen K E, Bruze M, Pirker C, Menne T. Hydroxyisohexyl 3-cyclohexene carboxaldehyde- known as Lyral: quantitative aspects and risk assessment of an important fragrance allergen. *Contact Dermatitis* 2003: 48: 310-316.

225. Rastogi S C, Johansen J D, Menne T. Natural ingredients based cosmetics. Content of selected fragrance sensitizers. *Contact Dermatitis* 1996: 34: 423-426.

226. Müller P M, Lamparsky D. *Perfumes: Art Science and Technology*. London: Elsevier Applied Science, 1991.

227. Poulsen P B, Schmidt A. *A survey and health assessment of cosmetic products for children. Survey of Chemical Substances in Consumer Products, No. 88*. Copenhagen: Danish Environmental Protection Agency, 2007.

228. Rastogi S C, Jensen G H, Johansen J D. *Survey and risk assessment of chemical substances in deodorants. Survey of Chemical Substances in Consumer Products, No. 86*. Copenhagen: Danish Environmental Protection Agency, 2007.

229. Buckley D A. Fragrance ingredient labelling in products on sale in the U.K. *Br J Dermatol* 2007: 157: 295-300.

230. Rastogi S C, Lepoittevin J P, Johansen J D, Frosch P J, Menne T, Bruze M, Dreier B, Andersen K E, White I R. Fragrances and other materials in deodorants: search for potentially sensitizing molecules using combined GC-MS and structure activity relationship (SAR) analysis. *Contact Dermatitis* 1998: 39: 293-303.

231. Rastogi S C, Johansen J D, Frosch P, Menne T, Bruze M, Lepoittevin J P, Dreier B, Andersen K E, White I R. Deodorants on the European market: quantitative chemical analysis of 21 fragrances. *Contact Dermatitis* 1998: 38: 29-35.

232. Rastogi S C, Menne T, Johansen J D. The composition of fine fragrances is changing. *Contact Dermatitis* 2003: 48: 130-132.

233. Rastogi S C, Johansen J D, Bossi R. Selected important fragrance sensitizers in perfumes--current exposures. *Contact Dermatitis* 2007: 56: 201-204.

234. Rastogi S C, Bossi R, Johansen J D, Menne T, Bernard G, Gimenez-Arnau E, Lepoittevin J P. Content of oak moss allergens atranol and chloroatranol in perfumes and similar products. *Contact Dermatitis* 2004: 50: 367-370.

235. Rastogi S C, Johansen J D, Menne T, Frosch P, Bruze M, Andersen K E, Lepoittevin J P, Wakelin S, White I R. Contents of fragrance allergens in children's cosmetics and cosmetic-toys. *Contact Dermatitis* 1999: 41: 84-88.

236. Rastogi S C. *Contents of selected fragrance materials in cleaning products and other consumer products. Survey of chemical compounds in consumer products, No. 8*. Copenhagen: Danish Environmental Protection Agency, 2002.

237. Bernard G, Gimenez-Arnau E, Rastogi S C, Heydorn S, Johansen J D, Menne T, Goossens A, Andersen K, Lepoittevin J P. Contact allergy to oak moss: search for sensitizing molecules using combined bioassay-guided chemical fractionation, GC-MS, and structure-activity relationship analysis. *Arch Dermatol Res* 2003: 295: 229-235.

238. Johansen J D, Andersen K E, Svedman C, Bruze M, Bernard G, Gimenez-Arnau E, Rastogi S C, Lepoittevin J P, Menne T. Chloroatranol, an extremely potent allergen hidden in perfumes: a dose-response elicitation study. *Contact Dermatitis* 2003: 49: 180-184.

239. SCCP. *Opinion on Atranol and Chloroatranol present in natural extracts (e.g. oak moss and tree moss extract). Scientific Committee on Consumer Products, adopted 7 December 2004*. 2004.

240. White I R, Johansen J D, Arnau E G, Lepoittevin J P, Rastogi S, Bruze M, Andersen K E, Frosch P J, Goossens A, Menne T. Isoeugenol is an important contact allergen: can it be safely replaced with isoeugenyl acetate? *Contact Dermatitis* 1999: 41: 272-275.

241. SCCP. *Opinion on Hydroxyisohexyl 3-cyclohexene carboxaldehyde (sensitisation only). Scientific Committee on Consumer Products. Adopted 7 December 2004*. 2004.

242. Nardelli A, D'Hooghe E, Drieghe J, Dooms M, Goossens A. Allergic contact dermatitis from fragrance components in specific topical pharmaceutical products in Belgium. *Contact Dermatitis* 2009: 60: 303-313.

243. Fisher A A. Cosmetic dermatitis in childhood. *Cutis* 1995: 55: 15-16.

244. Corea N V, Basketter D A, Clapp C, Van Asten A, Marty J P, Pons-Guiraud A, Laverdet C. Fragrance allergy: assessing the risk from washed fabrics. *Contact Dermatitis* 2006: 55: 48-53.

245. Hartmann K, Hunzelmann N. Allergic contact dermatitis from cinnamon as an odour-neutralizing agent in shoe insoles. *Contact Dermatitis* 2004: 50: 253-254.

246. Murphy L A, White I R. Contact dermatitis from geraniol in washing-up liquid. *Contact Dermatitis* 2003: 49: 52.

247. Foti C, Zambonin C G, Conserva A, Casulli C, D'Accolti L, Angelini G. Occupational contact dermatitis to a limonene-based solvent in a histopathology technician. *Contact Dermatitis* 2007: 56: 109-112.

248. Topham E J, Wakelin S H. D-Limonene contact dermatitis from hand cleansers. *Contact Dermatitis* 2003: 49: 108-109.

249. Wakelin S H, McFadden J P, Leonard J N, Rycroft R J. Allergic contact dermatitis from d-limonene in a laboratory technician. *Contact Dermatitis* 1998: 38: 164-165.

250. Rastogi S C, Heydorn S, Johansen J D, Basketter D A. Fragrance chemicals in domestic and occupational products. *Contact Dermatitis* 2001: 45: 221-225.

251. Yazar K, Johnsson S, Lind M L, Boman A, Liden C. Preservatives and fragrances in selected consumer-available cosmetics and detergents. *Contact Dermatitis* 2011: 64: 265-272.

252. Api A M, Bredbenner A, McGowen M, Niemiera D, Parker L, Renskers K, Selim S, Sgaramella R, Signorelli R, Tedrow S, Troy W. Skin contact transfer of three fragrance residues from candles to human hands. *Regul Toxicol Pharmacol* 2007: 48: 279-283.

253. Nadiminti H, Ehrlich A, Udey M C. Oral erosions as a manifestation of allergic contact sensitivity to cinnamon mints. *Contact Dermatitis* 2005: 52: 46-47.

254. Hoskyn J, Guin J D. Contact allergy to cinnamal in a patient with oral lichen planus. *Contact Dermatitis* 2005: 52: 160-161.

255. Silvestre J F, Albares M P, Blanes M, Pascual J C, Pastor N. Allergic contact gingivitis due to eugenol present in a restorative dental material. *Contact Dermatitis* 2005: 52: 341.

256. Guarneri F, Barbuzza O, Vaccaro M, Galtieri G. Allergic contact dermatitis and asthma caused by limonene in a labourer handling citrus fruits. *Contact Dermatitis* 2008: 58: 315-316.

257. Wallenhammar L M, Ortengren U, Adreasson H, Barregard L, Björkner B, Karlsson S, et al. Contact allergy and hand eczema in Swedish dentists. *Contact Dermatitis* 2000: 43: 192-199.

258. Geier J, Lessmann H, Schnuch A, Uter W. Contact sensitizations in metalworkers with occupational dermatitis exposed to water-based metalworking fluids: results of the research project "FaSt". *Int Arch Occup Environ Health* 2004: 77: 543-551.

259. Decapite T J, Anderson B E. Allergic contact dermatitis from cinnamic aldehyde found in an industrial odour-masking agent. *Contact Dermatitis* 2004: 51: 312-313.

260. Schubert H J. Skin diseases in workers at a perfume factory. *Contact Dermatitis* 2006: 55: 81-83.

261. Heydorn S, Andersen K E, Johansen J D, Menne T. A stronger patch test elicitation reaction to the allergen hydroxycitronellal plus the irritant sodium lauryl sulfate. *Contact Dermatitis* 2003: 49: 133-139.

262. Hannuksela M. Sensitivity of Various Skin Sites in the Repeated Open Application Test. *Am J Contact Dermatitis* 1991: 2: 102-104.

263. Fischer L A, Menné T, Avnstorp C, Kasting G B, Johansen J D. Hydroxyisohexyl 3-cyclohexene carboxaldehyde allergy: relationship between patch test and repeated open application test thresholds. *Br J Dermatol* 2009: 161: 560-567.

264. Andersen K E, Johansen J D, Bruze M, Frosch P J, Goossens A, Lepoittevin J P, Rastogi S, White I, Menne T. The time-dose-response relationship for elicitation of contact dermatitis in isoeugenol allergic individuals. *Toxicol Appl Pharmacol* 2001: 170: 166-171.

265. DeGroot A C, Frosch P J. Adverse reactions to fragrances. A clinical review. *Contact Dermatitis* 1997: 36: 57-86.

266. Schnuch A, Geier J, Uter W, Frosch P J. Another look on allergies to fragrances: frequencies of sensitisation to the fragrance mix and its constituents. Results from the IVDK. *Exog Dermatol* 2002: 1: 231-237.

267. Schnuch A, Uter W, Geier J, Lessmann H, Frosch P J. Contact allergy to farnesol in 2021 consecutively patch tested patients. Results of the IVDK. *Contact Dermatitis* 2004: 50: 117-121.

268. Uter W, Schnuch A, Gefeller O. Guidelines for the descriptive presentation and statistical analysis of contact allergy data. *Contact Dermatitis* 2004: 51: 47-56.

269. Uter W, Gefeller O, Geier J, Lessmann H, Pfahlberg A, Schnuch A. *Untersuchungen zur Abhängigkeit der Sensibilisierung gegen wichtige Allergene von arbeitsbedingten sowie individuellen Faktoren. Schriftenreihe der Bundesanstalt für Arbeitsschutz und Arbeitsmedizin, Forschung, Fb 949*. Bremerhaven: 2002.

270. van Loveren H, Cockshott A, Gebel T, Gundert-Remy U, de Jong W H, Matheson J, McGarry H, Musset L, Selgrade M K, Vickers C. Skin sensitization in chemical risk assessment: report of a WHO/IPCS international workshop focusing on dose-response assessment. *Regulatory toxicology and pharmacology : RTP* 2008: 50: 155-199.

271. Kimber I, Dearman R J, Basketter D A, Ryan C A, Gerberick G F, McNamee P M, Lalko J, Api A M. Dose metrics in the acquisition of skin sensitization: thresholds and importance of dose per unit area. *Regulatory toxicology and pharmacology : RTP* 2008: 52: 39-45.

272. Paramasivan P, Lai C, Pickard C, Ardern-Jones M, Healy E, Friedmann PS. Repeated low-dose skin exposure is an effective sensitizing stimulus, a factor to be taken into account in predicting sensitization risk. *Br J Dermatol.* 2010: 162: 594-7

273. Suskind R R. The hydroxycitronellal story: What can we learn from it? In: Frosch P J, Johansen J D, White I R, eds. *Fragrances Beneficial and adverse effects*. Berlin, Heidelberg, New York: Springer, 1988: 159-165.

274. Api A M, Basketter D, Cadby P A, Cano M-F, Ellis G, Gerberick F, Griem P, McNamee P M, Ryan C A, Safford B. *Dermal Sensitization Quantitative Risk Assessment (QRA) For Fragrance Ingredients Technical Dossier. June 22, 2006 QRA Expert Group.* http://www.ifraorg.org/en-us/search/tags_21261 *(last accessed 2011-11-27)*. 2006.

275. SCCP. Opinion on Dermal Sensitisation Quantitative Risk Assessment (Citral, Farnesol and Phenylacetaldehyde). Scientific Committee for on Consumer Protection, adopted 24 June 2008. 2008:

276. Fischer L A, Voelund A, Andersen K E, Menne T, Johansen J D. The dose-response relationship between the patch test and ROAT and the potential use for regulatory purposes. *Contact Dermatitis* 2009: 61: 201-208.

277. Roberts D W. QSAR for upper-respiratory tract irritation. *Chem Biol Interact* 1986: 57: 325-345.

278. Roberts D W, Natsch A. High throughput kinetic profiling approach for covalent binding to peptides: application to skin sensitization potency of Michael acceptor electrophiles. *Chem Res Toxicol* 2009: 22: 592-603.

279. Bruze M, Johansen J D, Andersen K E, Frosch P, Goossens A, Lepoittevin J P, Rastogi S C, White I, Menne T. Deodorants: an experimental provocation study with isoeugenol. *Contact Dermatitis* 2005: 52: 260-267.

280. Fischer L A, Menne T, Voelund A, Johansen J D. Can exposure limitations for well-known contact allergens be simplified? An analysis of dose-response patch test data. *Contact Dermatitis* 2011: 64: 337-342.

281. Franot C, Roberts D W, Basketter D A, Benezra C, Lepoittevin J P. Structure-activity relationships for contact allergenic potential of gamma,gamma-dimethyl-gamma-butyrolactone derivatives. 2. Quantitative structure-skin sensitization relationships for alpha- substituted-alpha-methyl-gamma,gamma-dimethyl-gamma-butyrolactone s. *Chem Res Toxicol* 1994: 7: 307-312.

282. SCCS. *Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation, 7th revision . Scientific Committee for Consumer Safety, adopted 14 December 2010*. 2010.

283. Basketter D, Horev L, Slodovnik D, Merimes S, Trattner A, Ingber A. Investigation of the threshold for allergic reactivity to chromium. *Contact Dermatitis* 2001: 44: 70-74.

284. Pasche F, Hunziker N. Sensitization to Kathon CG in Geneva and Switzerland. *Contact Dermatitis* 1989: 20: 115-119.

285. Fischer L A, Johansen J D, Menne T. Nickel allergy: relationship between patch test and repeated open application test thresholds. *Br J Dermatol* 2007: 157: 723-729.

286. Fischer L A, Johansen J D, Menne T. Methyldibromoglutaronitrile allergy: relationship between patch test and repeated open application test thresholds. *Br J Dermatol* 2008: 159: 1138-1143.

287. Flyvholm M A, Hall B M, Agner T, et al. Threshold for occluded formaldehyde patch test in formaldehyde- sensitive patients. Relationship to repeated open application test with a product containing formaldehyde releaser. *Contact Dermatitis* 1997: 36: 26-33.

288. Thyssen J P, Johansen J D, Menne T, Nielsen N H, Linneberg A. Nickel allergy in Danish women before and after nickel regulation. *The New England journal of medicine* 2009: 360: 2259-2260.

289. Thyssen J P, Linneberg A, Menne T, Nielsen N H, Johansen J D. The association between hand eczema and nickel allergy has weakened among young women in the general population following the Danish nickel regulation: results from two cross-sectional studies. *Contact Dermatitis* 2009: 61: 342-348.

290. Zachariae C O, Agner T, Menné T. Chromium allergy in consecutive patients in a country where ferrous sulfate has been added since 1981. *Contact Dermatitis* 1996: 35: 83-85.

291. Braendstrup P, Johansen J D. Hydroxyisohexyl 3-cyclohexene carboxaldehyde (Lyral) is still a frequent allergen. *Contact Dermatitis* 2008: 59: 187-188.

292. SCCNFP. *Opinion on hydroxyisohexyl 3-cyclohexene carboxaldehyde. The Scientific Committee on Cosmetic products and Non-Food Products Intended for Consumers,adopted 9 December 2003*. 2003.

293. Api A M, Vey M. A new IFRA Standard on the fragrance ingredient, hydroxyisohexyl 3-cyclohexene carboxaldehyde. *Contact Dermatitis* 2010: 62: 254-255.

294. Schnuch A, Geier J, Uter W. Is hydroxyisohexyl 3-cyclohexene carboxaldehyde sensitisation declinging in central Europe? *Contact Dermatitis* 2012: 67: 47-49.

295. Heisterberg M V, Laurberg G, Veien N, Menné T, Avnstorp C, Kaaber K, Andersen K A, Sommerlund M, Danielsen A, Andersen B, Kristensen B, Kristensen O, Nielsen N H, Thormann J, Vissing S, Johansen J D. Prevalence of allergic contact dermatitis caused by hydroxyisohexyl 3-cyclohexene carboxaldehye has not changed in Denmark. *Contact Dermatitis* 2012: 67: 49-51.

296. Nardelli A, Gimenez-Arnau E, Bernard G, Lepoittevin J P, Goossens A. Is a low content in atranol/chloroatranol safe in oak moss-sensitized individuals? *Contact Dermatitis* 2009: 60: 91-95.

297. Anonymous. *OECD Guidelines for the Testing of Chemicals / Section 4: Health Effects. Test No. 429: Skin Sensitisation (Local Lymph Node Assay)*. Paris: OECD, 2002.

298. Anonymous. 76/768/EEC - Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products. *Official Journal L* 1976: 262, 27/09/1976: 169.

299. Vigan M. Contact dermatitis sentinel network by GERDA. *Nouv Dermatol* 1996: 15: 677-678.

300. SCCP. *Opinion on Oak moss/Tree moss (sensitisation only) Scientific Committee on Consumer Products, adopted 15 April 2008*. 2008.

Annex I - Catalgoue of fragrance allergens

Contents

Single chemicals

Often, results with the single constituents of the FM I or, yet more rarely, FM II, are presented in one paper. As the main ordering of this annex is by allergen, core information on these studies is presented in a tabular format and referenced by a unique acronym in the single sections, to avoid redundancy. Regarding nomenclature, terms which are often not officially an INCI Name but Perfuming Name as listed by CosIng are used. “Current Regulation” refers to the EU Cometics Directive only.

Table 55: Background information on studies reporting results with (all) single constituents of the FM I (**amyl cinnamal, cinnamyl alcohol, cinnamal, eugenol, geraniol, hydroxycitronellal, isoeugenol, EVERNIA PRUNASTRI**)

Reference	Country	Study period, Patients	Comments by reviewers
Larsen 2002 c (1)	7 industrial countries worldwide	Prior to 2002 n=218 patients with known contact allergy to fragrance ingredients	Test concentrations identified as non-irritating in serial dilution testing in 20 healthy volunteers
Utrecht 1999 (2)	Utrecht, The Netherlands	1994-1998 n=757 patients with suspected ACD to cosmetics	All patients tested with FM I and single constituents
Sheffield 1999 (3)	UK	1994-1995 n=744, 40 of these positive to FM I and tested with single constituents	
IVDK 2007 (4)	Germany + one centre in Austria and Switzerland each	01/2003 – 12/2004, n=1658 to 21325, see text, consecutive patients	
Hungary 2002 (5)	Hungary, multicentre study,	1998-1999, n=3604 patients	recruitment not clear, presumably consecutive patients
Groningen 2009 (6)	Groningen, The Netherlands	04/2005-06/2007 n=320	patients selected according to history or site suspicious of contact allergy to fragrance ingredients
IVDK 2010 (7)	Germany, Switzerland and one centre in	2005-2008 n=36961 tested with FM I, n=4167 with FM II and	

	Austria	all SC	

Table 56: Results of PTing with single constituents of the FM I in patients positive to the FM I (as percent)

N(pos) to FM I, ref.	*Evernia prun.*	Isoeug.	Hydroxy citron.	Cinnamal	Cinnamyl alcohol	Eugenol	Gera-niol	Alpha-amyl cinnamal
N=160 (5)	13.1%	14.8 %	2.5%	8.1%	20.6%	8.8%	7.5%	5.0%
N= 991 (8)	18.4%	11.2 %	10.1%	6.1%	6.1%	6.6%	4.6%	2.4%
N=50 (2)	19.6%	14.3 %	8.9%	8.9%	7.1%	5.4%	2.7%	0%
n=40 Sheffield 1999 (3)	30%	20%	2.5%	12.5 %	10%	5%	0%	0%
N=226 Coimbra 2000 (9)	22.1%	19.9 %	6.6%	13.3 %	7.9%	14.6 %	8.4%	4.4%
N=655 IVDK 2010 (7)	29.8%	18.0 %	12.8%	11.6 %	9.6%	6.7%	4.7%	2.8%

Table 57: Background information on studies reporting results with (all) single constituents of the FM II (**citronellol, citral, coumarin, hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), Farnesol, alpha-Hexyl-cinnamic aldehyde**)

Reference	Country	Study period, Patients	Comments by reviewers
IVDK 2007 (4)	Germany + one centre in Austria and Switzerland each	01/2003 – 12/2004, n=1658 to 21325, see text, consecutive patients	
EU 2005 (10)	6 European centres	10/2002 – 06/2003, n=1701	Applied in consecutive patients
Groningen 2009 (6)	Groningen, The Netherlands	04/2005-06/2007 n=320	patients selected according to history or site suspicious of contact allergy to fragrance ingredients

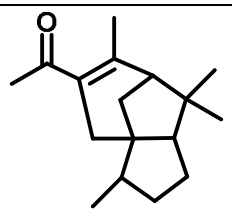
IVDK 2010b (11)	Germany, Switzerland and one centre in Austria	2005-2008 n=35633 tested with FM II, n=2217 with all SC	

Table 58: Background information on studies reporting results with several fragrance compounds not, or only partly, corresponding to mixes (later created) or with essential oils

Reference	Country	Study period, Patients
deGroot 2000 (12)	The Netherlands (multicentre)	09/1998-04/1999 n=1825 consecutive patients
An 2005 (13)	South Korea (multicentre)	04/2002 – 06/2003 n=422 consecutive patients
Sugiura 2000 (14)	Nagoya, Japan	1990-1998 n=1483 patients with suspected cosmetic dermatitis
Frosch 1995 (15)	11 European depts.	Prior to 1995 n=1069 consecutive patients
Frosch 2002 a (16)	6 European depts.	10/1997-10/1998 n=1855 consecutive patients
Frosch 2002 b (17)	6 European depts.	Prior to 2002 n=1606 consecutive patients
Coimbra 2000 (9)	Portugal	07/1989-06/1999 n=226 with FM I SC n=67 also with other fragrances
Larsen 1977 (18)	US	1977 n=20 “perfume-sensitive patients”
Larsen 2001 (19)	worldwide multicentre	? (prior to 2001) n=178 patients with known contact allergy to fragrance ingredients
Belsito 2006 (20)	North American (5 US, 1 Canadian) depts.	2003 n=1603 patients
NACDG 2009 (21)	US and Canada	2005-2006 n= 4454 patients
Wöhrl 2001 (22)	“FAZ” clinic Vienna	1997-2000 n=747 of 2660 consecutive patients tested with special series
EECDRG 1995 (15)	European, multicentre	Different fragrances, tested in 2 concentrations, in sets of about 100 patients each in different centres
Goossens 1997 (23)	Leuven, Belgium	1978-1987 n=111 “Japanese perfume series” (highly selected patients)

Reference	Country	Study period, Patients
Malten 1984 (24)	Dutch multicentre	N=182 patients with suspected cosmetic dermatitis tested with 22 fragrance compounds
DeGroot 1985 (25)	Dutch	N=179 patients with suspected cosmetic dermatitis tested with 16 fragrance compounds
Rudzki 1976 (26)	Warsaw, Poland	N=200 consecutive patients
Rudzki 1986 (27)	Warsaw, Poland	N=86 patients of 299 (of 5315) patients with positive reaction to FM I tested with essential oils series
Santucci 1987 (28)	Rome, Italy	N=1500 consecutive patients; n=63 reacting positively to FM I re-tested with extended fragrance series
Nakayama 1974 (after (29))	Japan	N=183 patients with cosmetic dermatitis
IVDK 2010c (30)	Germany, Switzerland and one centre in Austria	15682 patients tested with at least one essential oil in different test series
Trattner/David (31)	Tel Aviv, Israel	N=641 consecutive patients

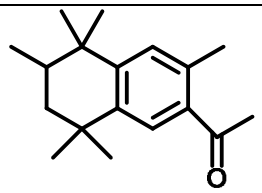
Catalogue of single chemicals evaluated

ACETYLCEDRENE	
CAS # 32388-55-9	
EC # 251-020-3	
1-[(3R,3aR,7R,8aS)-2,3,4,7,8,8a-Hexahydro-3,6,8,8-tetramethyl-1H-3a,7-methanoazulen-5-yl]-ethanone	
Other names 1-(2,3,4,7,8,8a-Hexahydro-3,6,8,8-tetramethyl-1H-3a,7-methanoazulen-5-yl)-, [3R-(3α,3aβ,7β,8aα)]-Ethanone; 1H-3a,7-Methanoazulene, Ethanone deriv.; Acetyl-α-cedrene; Lixetone; Vertofix	

Current regulation: /

Clinical data:
In the Frosch 2002 a study, a total of 0.2% had positive PT reactions (16). In the Frosch 1995 dose-finding pilot study, 1 positive reaction to 1% and and none to 5% “Vertofix ®” in pet., tested in 100 consecutive patients in Stockholm, were observed (15). In a case report, a 28-year-old patient with axillary dermatitis after using 2 different deodorants tested positive not only to HICC, but also to acetyl cedrene (tested 10.8% in diisopropylene glycol (20 healthy controls negative) (32). In this case report it is stated that “Acetyl cedrene (Vertofix Coeur) is a complex reaction mixture of which a principal constituent is methyl cedryl ketone”.

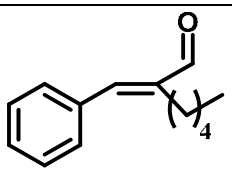
Additional information:
Acetyl cedrene (Vertofix®, IFF) is a complex mixture obtained from cedar wood oil by the acetylation of terpenes. The principal component of acetyl cedrene is methyl cedryl ketone (CAS 32388-55-9). It is a “top 100” substance (IFRA, pers. comm.2010)

6-ACETYL-1,1,2,4,4,7-HEXAMETHYLTETRALINE	
CAS # 21145-77-7	
EC # 216-133-4 / 244-240-6	
1-(5,6,7,8-Tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthalenyl)-ethanone	
AHMT (perfume), AHTN, Extralide, Fixolide, Musk tonalid, NSC 19550, Tentarome, Tetralide, Tonalid, Tonalide.	

Current regulation: Annex III, part 1, entry 182

Clinical data:
In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% “Tonalide ®” in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15).

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

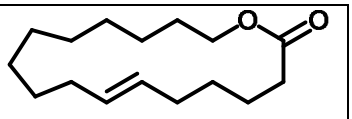
AMYL CINNAMAL	
CAS # 122-40-7	
EC # 204-541-5	
2-(Phenylmethylene)-heptanal	
Cinnamaldehyde, α-amyl- (4CI); Cinnamaldehyde, α-pentyl- (6CI,7CI,8CI); 2-(Phenylmethylene)heptanal; 2-Benzylideneheptanal; Amylcinnamaldehyde; Amylcinnamic acid aldehyde; Amylcinnamic aldehyde; Flomine; Jasminal; Jasminaldehyde; Jasmine aldehyde; NSC 6649; Pentylcinnamaldehyde; α-Amyl-β-phenylacrolein; α-Amylcinnamal; α-Amylcinnamaldehyde; α-Pentylcinnamaldehyde	

Current regulation: Annex III, part 1, entry 67

Clinical data:
In the "background information" section of the 1999 opinion (33), amyl cinnamal (synonymous: alpha amyl cinnamaldehyde) has been classified as frequently reported contact allergen because it has been identified as a cause of allergic reactions in persons with eczema from cosmetic products.

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded n=4, i.e., 0.2% (95% CI: 0.1 – 0.5%) positive reactions to this compound (1% pet.) in 2062 consecutively PTed patients (4). In the Groningen 2009 study, no positive reactions to this allergen, tested at 2% pet., were observed (6). The Larsen 2001 study yielded 2.3% positive reactions in 178 patients with known contact allergy to fragrance ingredients (test concentration: 5% pet.) (19). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=2 (0.3%) positive reactions to amyl cinnamal (22). The IVDK 2010 study, 0.26% (95% CI: 0 – 0.60%) of 1214 consecutively tested patients reacted to the compound, while 0.61% (95% CI: 0.36 – 0.86%) of 4375 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7).

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

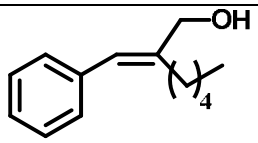
AMBRETTOLIDE	
CAS # 7779-50-2	
EC # 231-929-1	
Oxacycloheptadec-7-en-2-one	
1-Oxa-7-cycloheptadecen-2-one; 16-Hydroxy-6-hexadecenoic acid lactone; 16-Hydroxy-6-hexadecenoic acid ω-lactone	

Current regulation: /

Clinical data:
The Larsen 2001 study, using omega-6-hexadecenlactone (HDL, 5% pet.) as test concentration, diagnosed 3.4% positive reactions in 178 patients with known contact allergy to fragrance ingredients (19).

Additional information:

Ambrettolide is 1 of 2 components of Ambrette seed oil (obtained from *Hibiscus abelmoschus* L., *Malvaceae*) responsible for the musk odour. In Surburg/Panten, the compound has the chemical name (Z)-7-hexadecen-16-olide (or Hexadec-7-en-16-olide according to CosIng), CAS 123-69-3 (34).

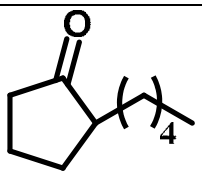
AMYL CINNAMYL ALCOHOL	
CAS # 101-85-9	
EC # 202-982-8	
2-(Phenylmethylene)-heptan-1-ol,	
2-Benzylidene- (6CI,8CI)1-heptanol; 2-Amyl-3-phenyl-2-propen-1-ol; 2-Benzylidene-1-heptanol; 2-Pentyl-3-phenyl-2-propen-1-ol; Buxinol; α-Amylcinnamic alcohol; α-Amylcinnamyl alcohol	

Current regulation: Annex II, Part 1, entry 74

Clinical data:
In the "background information" section of the 1999 opinion, amyl cinnamyl alcohol is mentioned to cross-react with amyl cinnamal. Moreover, this compound has been identified as a cause of allergic reactions in a notable number of persons with eczema from the use of cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.4% (95% CI: 0.1 – 0.7%) positive reactions in 1977 consecutively PTed patients (4). The IVDK 2010 study, 0.79% (95% CI: 0.54 – 1.04%; percentages standardised for age and sex) of 5650 patients PTed reacted to the compound (7). In the Groningen 2009 study, 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen (6).

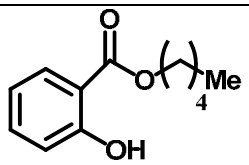
Additional information: A RIFM review is available (35) where selected clinical studies published until 1994 were considered.

AMYLCYCLOPENTANONE	
CAS # 4819-67-4	
EC # 225-392-2	
2-Pentylcyclopentanone	
2-Pentyl-1-cyclopentanone; 2-Pentylcyclopentanone; 2-Pentylcyclopenten-1-one; 2-n-Amylcyclopentanone; 2-n-Pentyl cyclopentanone; Delphone	

Current regulation: /

Clinical data:
In the Larsen 2001 study, none of 178 patients with contact allergy to fragrance ingredients reacted positively to this ingredient, PTed at 5% pet. (19).

Additional information: /

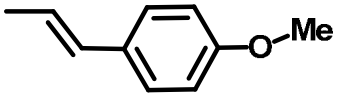
AMYL SALICYLATE	
CAS # 2050-08-0	
EC # 218-080-2	
Pentyl-2-hydroxybenzoate	
Amyl ester salicylic acid, (4CI); Pentyl ester salicylic acid, (6CI,8CI); 2-Hydroxybenzoic acid pentyl ester; Amyl salicylate; NSC 403668; NSC 44877; NSC 46125; Pentyl salicylate	

Current regulation: /

Clinical data:

In the Frosch 2002 a study, a total of n=3 (0.2%) had positive PT reactions (16). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% amyl salicylate and 1 positive reaction to 5% amyl salicylate were observed in 100 consecutive patients patch tested in Stockholm (15).

Additional information:
A RIFM review is available (36). It is a “top 100” substance (IFRA, pers. comm.2010)

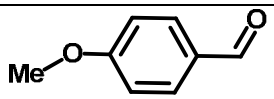
trans-ANETHOLE	
CAS # 4180-23-8	
EC # 224-052-0 / 203-205-5	
1-Methoxy-4-(1E)-1-propen-1-yl-benzene	
(E)-p-Propenyl-anisole (8CI); (E)-1-Methoxy-4-(1-propenyl)-benzene; 1-Methoxy-4-(1E)-1-propenyl-benzene (9CI); (E)-1-(4-Methoxyphenyl)propene; (E)-1-p-Methoxyphenylpropene; (E)-Anethol; (E)-Anethole (REACH, EINECS); *E*-Anethole (INCI); 1-Methoxy-4-[(1E)-1-propenyl]benzene; (E)-1-Methoxy-4-(1-propenyl)-benzene (CosIng); NSC 209529; trans-1-(4-Methoxyphenyl)-1-propene; trans-1-(p-Methoxyphenyl)-1-propene; trans-1-(p-Methoxyphenyl)propene; trans-1-p-Anisylpropene; trans-4-(1-Propenyl)anisole; trans-Anethol; trans-Anethole; trans-p-Anethole; trans-p-Methoxy-β-methylstyrene	

Current regulation: /

Clinical data:
A case of a 64 year old patient, who developed severe cheilitis and a loss of taste has been described (37). Both were reversible after the cessation of use of previous toothpastes. The patch test was strongly positive to anethole (isoform not given) 5% pet.; this was found an ingredient of the causative toothpaste. Two cases of occupational allergic contact dermatitis occurring in a traditional cake factory due to anise oil have been described, both testing (strongly) positive to anise oil (5% o.o.) and anethole (5% pet.) (38).

Additional information:
It is a “top 100” substance (IFRA, pers. comm.2010). trans-Anethole can be purified from star anise oil (34, 39), see 3.2., and is the main component of anise, star anise

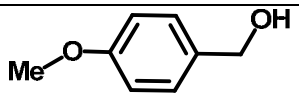
and fennel oils (38)

ANISALDEHYDE	
CAS # 123-11-5	
EC # 204-602-6	
4-Methoxy-benzaldehyde	
p-Methoxybenzaldehyde; p-Anisaldehyde; 4-Anisaldehyde; Aubepine; Crategine; NSC 5590; Obepin; p-Anisic aldehyde; Anisic aldehyde; p-Formylanisole.	

Current regulation: /

Clinical data: /

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

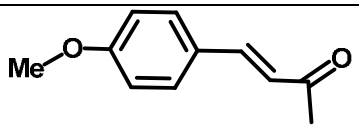
ANISYL ALCOHOL	
CAS # 105-13-5	
EC # 203-273-6	
4-Methoxy-benzenemethanol	
p-Methoxy-benzyl alcohol (8CI); (4-Methoxyphenyl)methyl alcohol; 4-(Hydroxymethyl)anisole; 4-(Methoxyphenyl)methanol; 4-Methoxy-α-hydroxytoluene; 4-Methoxybenzenemethanol; 4-Methoxybenzyl alcohol; Anise alcohol; Anisic alcohol; NSC 2151; [4-(Methyloxy)phenyl]methanol; p-(Methoxyphenyl)methanol; p-Anisalcohol; p-Anisyl alcohol; p-Methoxybenzyl alcohol	

Current regulation: Annex III, part 1, n° 80

Clinical data:
In the “background information” section of the 1999 opinion, anisyl alcohol is classified as “less frequently reported allergen”; 2 studies were identified where 3 and 4 cases, respectively, with cosmetic dermatitis due to contact allergy to anisyl alcohol had been reported (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded n=1, i.e., 0.1% (95% CI: 0.00 – 0.3%) positive reactions in 2004 consecutively PTed patients, patch test concentration: 1% pet. (4). Similar results were obtained in the following period, with n=1 (and n=3 irritant and n=6 doubtful) reactions in 986 patients tested with 1% in pet. (30). In the Groningen 2009 study, no positive reactions to this allergen, tested at 5% pet., were observed in 320 patients (6).This test concentration has been regarded as relatively high by Hostynek and Maibach (40). The test concentration of Anisyl Alcohol has been further validated by Bruze et al. and 10% in pet was recommended as a non-irritant concentration for routine investigations (40a).

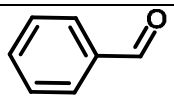
Additional information: /

ANISYLIDENE ACETONE	
CAS # 943-88-4	
EC # 213-404-9	
4-(4-Methoxyphenyl)-3-Buten-2-one	
1-(p-Methoxyphenyl)-1-buten-3-one; 4-(4-Methoxyphenyl)-3-buten-2-one; 4-(p-Methoxyphenyl)-3-buten-2-one; 4-Methoxybenzalacetone; 4-Methoxybenzylideneacetone; 4-Methoxystyryl methyl ketone; 4'-Methoxybenzylideneacetone; Anisalacetone; Methyl p-methoxystyryl ketone; NSC 31752; NSC 7946; p-Anisalacetone; p-Methoxybenzalacetone; p-Methoxybenzylideneacetone; p-Methoxystyryl methyl ketone	

Current regulation: Annex III, part 1, n° 443

Clinical data:
In the Malten 1984 study, 1.1% of 182 patients displayed a positive PT reaction to anisylidene acetone 2% pet. (24)

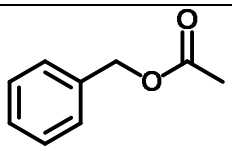
Additional information: /

BENZALDEHYDE	
CAS # 100-52-7	
EC # 202-860-4	
Benzaldehyde	
Artificial Almond Oil; Benzaldehyde FFC; Benzenecarbonal; Benzenecarboxaldehyde; Benzoic acid aldehyde; Benzoic aldehyde; NSC 7917; Phenylformaldehyde; Phenylmethanal	

Current regulation: /

Clinical data:
In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=3 (0.4%) positive reactions to benzaldehyde 5% pet. (22). The IVDK 2010 study, 6 weak positive reactions were observed, i.e., 0.16% (95% CI: 0.03 – 0.29%; percentages standardised for age and sex) of 2820 patients PTed reacted to the compound (7). A review is available in the Int. J. Toxicol. (41). In the case of a 19 year old pastry maker, Seite-Bellezza et al. report on immediate reactions to MP, cinnamal and benzaldehyde (tested at 5% pet.) subsiding after a few hours, in line with the patient's history (42).

Additional information: /

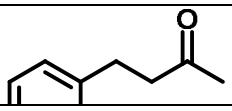
BENZYL ACETATE	
CAS #140-11-4	
EC # 205-399-7 / 202-940-9	
Benzyl actetate	
Benzyl ester acetic acid; Benzyl alcohol, acetate (6CI); (Acetoxymethyl)benzene; Benzyl ethanoate; NSC 4550; Phenylmethyl acetate; Methyl Phenylacetate; α-Acetoxytoluene ; Methyl alpha-Toluate	

Current regulation: /

Clinical data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% benzyl aceteate in pet., tested in 100 consecutive patients in Odense, DK, were observed (15). Benzyl acetate is a component of several natural mixtures, for example a major constituent of Narcissus abs., and a minor constituent of Jasmine abs. (17).

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

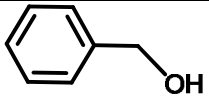
BENZYL ACETONE	
CAS # 2550-26-7	

EC # 219-847-4	
4-Phenyl-2-butanone	
4-Phenylbutan-2-one (REACH, EINECS); Benzylacetone; Methyl 2-phenylethyl ketone; Methyl phenethyl ketone; NSC 44829; NSC 813M; Phenethyl methyl ketone; 1-Phenyl-3-butanone; 2-Phenylethyl methyl ketone	

Current regulation: /

Clinical data: /

Additional information:
It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (43).

BENZYL ALCOHOL	
CAS # 100-51-6	
EC # 202-859-9	
Phenylmethanol	
Benzyl alcohol; (Hydroxymethyl)benzene; Benzenecarbinol; Benzylic alcohol; NSC 8044; Phenylcarbinol; Benzenemethanol; Phenylmethyl alcohol; Sunmorl BK 20; TB 13G; α-Hydroxytoluene; α-Toluenol	
Current regulation: Annex III, part 1, n° 45; Annex VI, part1, n ° 34	

Clinical data:
In the “background information” section of the 1999 opinion, benzyl alcohol is classified as allergen frequently causing allergic reactions. It has been found to cause allergic reactions in 1.2 to 15% of patients with eczema from cosmetic products (33). A CIR expert panel review is available in the Int. J. Toxicol. (44).

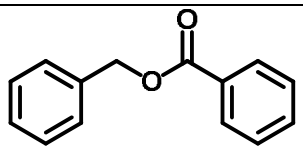
Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.3% (95% CI: 0.1 – 0.7%) positive reactions in 2166 consecutively PTed patients (4). In the Groningen 2009 study, n=1, i.e. 0.3% (95% CI: 0.01 – 1.7%) had positive reactions to this allergen (6).

Both in terms of case reports (45-47) and clinical epidemiology data (0.22 % [95% CI: 0.16 – 0.28%] positive tested with benzyl alcohol in the context of a “topical drugs” series, n=26448 (7)) the relevance of this alternative exposure is highlighted. In a study from Alicante, Spain, 86 selected patients were tested with benzyl alcohol, yielding 2 positive reactions (48).

After application of saline soaks preserved with benzyl alcohol onto his stasis dermatitis, a 53 year old patient developed a rash, which was, according to test results obtained by J. D. Guin and J. Goodman, at least partly due to an immediate hypersensitivity to benzyl alcohol, as verified by an intense urticarial reaction at the test site lasting several days (49). According to 2 cases reported by A. A. Fisher, PT-proven, relevant delayed type hypersensitivity is not associated with immediate reactions in scratch or intradermal tests (50). D. W. Shaw describes a patient with allergic contact dermatitis caused by benzyl alcohol in a hearing aid impression material and in topical medications (51). Another contribution points to covert exposures to benzyl alcohol even in products labelled “fragrance free” (52) probably because benzyl alcohol is used as preservative, or an essential oil containing benzyl alcohol is used as cosmetic ingredient.

Additional information:

Benzyl alcohol is a component of several natural mixtures, including Myroxylon pereirae resin, which have been used for extraction, but is nowadays synthesised (53). It is permitted in certain foodstuffs (liquors: < 100 mg/l, sweets and cakes: < 250 mg/kg) under the coding "E 1519" (http://www.zusatzstoffe-online.de/zusatzstoffe/317.e1519_benzylalkohol.html, last accessed 2009-11-27). In addition to being a fragrance compound (which may be used, even in relatively high concentration, to scent topical medications (54)), benzyl alcohol is used as antioxidant in topical therapeutics or cosmetics. The German "Rote Liste" (http://www.rote-liste.de, last accessed 2009-11-11), for instance, lists 205 specialties containing benzyl alcohol. Benzyl alcohol may be used up to 1.0% as a preservative in cosmetic products according to the Cosmetic Directivr 76/768/EEC

BENZYL BENZOATE	
CAS # 120-51-4	
EC # 204-402-9	
Benzyl benzoate	
Benzyl ester benzoic acid; Ascabin; Ascabiol; Benylate; Benzyl benzenecarboxylate; Benzyl benzoate; Benzyl phenylformate; Benzylets; Colebenz; NSC 8081; Nicca Sunsolt LM 7EX; Novoscabin; Pelemol B66; Peruscabin; Phenylmethyl benzoate; Scabagen; Scabanca; Scabcare BB; Scabide; Scabiozon; Scobenol; Vanzoate; Venzonate	

Current regulation: Annex III, part 1, n° 85

Clinical data:

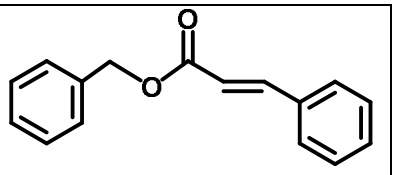
In the "background information" section of the 1999 opinion, benzyl benzoate is classified as "less frequently reported allergen"; in several studies, only single cases had been reported in each, except for patients sensitive to MP (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded n=1, i.e., 0.1% (95% CI: 0.00 – 0.3%) positive reactions in 2003 consecutively PTed patients, test concentration 1% pet. (4). In the subsequent period (2005-2008), n=1062 patients were tested in the IVDK 2010 study, with no positive reactions (7). In the Groningen 2009 study, no positive reactions to this allergen, tested at 5% pet., were observed in 320 patients (6). Thus, the pooled proportion of positive patch test reactions is 1 / 3385 (0.03%, exact upper 1-sided 95% CI: 0.14%)

Additional information:

Benzyl benzoate naturally occurs in MP resin and ylang-ylang oil. Nowadays it is synthesised and used for a variety of purposes (53). These include use as a scabicide (one brand specialty on the German market, using a concentration of 10% for children and 25% for adults), possibly with some differences among European countries. In France, a combination of benzyl benzoate 10% and sulfiram 2% is reported to be used most often (55). Hausen et al. review the older literature and mention a study identifying 1 sensitised patient in 73 patients treated for scabies (details not given) (53). According to the mandatory factsheet (see PDF "benzylbenzoate_infosheet_DE.pdf") dermatitis after anti-scabies treatment is "rare", in a range between 1:1000 and 1:10000.

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

BENZYL CINNAMATE	
CAS # 103-41-3	
EC # 203-109-3	
Benzyl 3-phenylprop-2-enoate	
Benzyl ester cinnamic acid; 3-phenyl-phenylmethyl ester 2-propenoic acid; 3-Phenyl-2-propenoic acid benzyl ester; Benzyl 3-phenylpropenoate; Benzyl γ-phenylacrylate; Cinnamein; NSC 11780; NSC 44403	

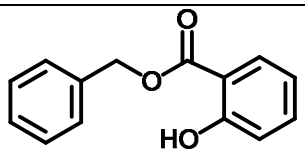
Current regulation: Annex III, part 1, n° 81

Clinical data:

In the “background information” section of the 1999 opinion, benzyl cinnamate (synonymous: benzyl 3-phenyl-2-propenoate, cinnamein) is classified as “less frequently reported allergen”; one study of patients with contact allergy to cosmetic products was identified and further a study where benzyl cinnamate associated with contact sensitisation to MP (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.3% (95% CI: 0.1 – 0.6%) positive reactions in 2042 consecutively PTed patients, test concentration 5% pet. (4). The IVDK 2010 study, n=4 weak positive were observed, amounting to 0.12% (95% CI: 0 – 0.25%; percentages standardised for age and sex) of 2872 patients PTed reacted to the compound (7). In the Groningen 2009 study, no positive reactions to this allergen, using the same test concentration, were observed in 320 patients (6). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=3 (0.4%) positive reactions (22).

Additional information: A RIFM review is available (56).

BENZYL SALICYLATE	
CAS # 118-58-1	
EC # 204-262-9	
Benzyl 2-hydroxybenzoate	
Salicylic acid, Benzyl ester; Benzoic acid, 2-Hydroxy-, phenylmethyl ester; Benzyl o-hydroxybenzoate; NSC 6647	

Current regulation: Annex III, part 1, n° 75

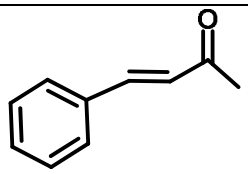
Clinical data:

In the "background information" section of the 1999 opinion (33), benzyl salicylate is classified among the frequent allergens, with 0.2 to 10% of patients with eczema from cosmetic products testing positively. In one study, benzyl salicylate accounted for 75% of reactions to commercial products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded n=2, i.e. 0.1% (95% CI: 0.01 – 0.4%) positive reactions in 2041 consecutively PTed patients (test concentration 1% pet.) (4). The IVDK 2010 study, 2 of 3775 patients PTed reacted weakly positive to the compound (7). In the Groningen 2009 study, n=1, i.e. 0.3% (95% CI: 0.01 – 1.7%) had positive reactions to this allergen, tested at 2% pet. (6). In the deGroot 2000 study, 10 of 1825 consecutive patients tested positive to benzyl salicylate (2% pet.), of these, 3 were not detected by the FM I (12). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=3 (0.4%) positive reactions (22). Trattner/David found 2 positive cases in 641 consecutive eczema patients (31). In a study from Alicante, Spain, 86 selected patients were tested with benzyl salicylate, yielding 2 positive reactions (48).

Additional information:

It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010). A RIFM review is available, including internal results on, e.g. HRIPT, and a review of LLNA results, where benzyl salicylate is classified as "weak" allergen (57).

BENZYLIDENEACETONE	
CAS # 122-57-6	
EC # 204-555-1	
4-Phenyl-3-buten-2-one	
4-Phenylbut-3-en-2-one; 2-Butenone, 4-Phenyl- (2CI); Ketone, Methyl styryl (7CI); 1-Phenyl-1-buten-3-one; 2-Phenylethenyl methyl ketone; 2-Phenylvinyl methyl ketone; 4-Phenyl-3-buten-2-one; 4-Phenyl-3-butene-2-one; 4-Phenylbutenone; Acetocinnamone; Benzalacetone; Benzylideneacetone; Methyl 2-phenylvinyl ketone; Methyl phenylvinyl ketone; Methyl styryl ketone; Methyl β-styryl ketone; NSC 5605; Styryl methyl ketone	

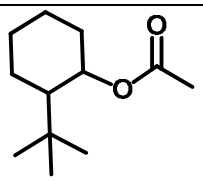
Current regulation: Annex II, n° 356

Clinical data:

In the Malten 1984 study, none of 182 patients displayed a positive PT reaction to

benzylidene acetone 0.5% pet. (24).

Additional information: /

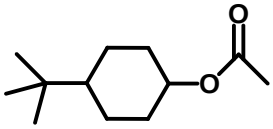
2-TERT-BUTYLCYCLOHEXYL ACETATE	
CAS # 88-41-5	
EC # 201-828-7	
2-(1,1-dimethylethyl)cyclohexyl acetate	
Cyclohexanol, 2-(1,1-dimethylethyl)-, acetate ; Cyclohexanol, 2-Tert-butyl-, acetate; 2-Tert-Butylcyclohexanol acetate; Verdox; o-Tert-Butylcyclohexyl acetate	

Current regulation: /

Clinical data:
In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% “Verdox ®” in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15)

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (58).

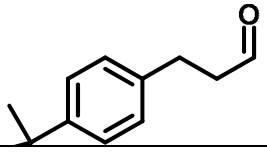
4-TERT-BUTYLCYCLOHEXYL ACETATE	
CAS # 32210-23-4	
EC # 250-954-9	
4-(1,1-Dimethylethyl)cyclohexyl acetate	
Boisinol A 464D; Cyclohexanol, 4-tert-Butyl-, acetate; Cyclohexanol, 4-(1,1-Dimethylethyl)-, acetate; 4-(1,1-Dimethylethyl)cyclohexyl acetate; 4-tert-Butylcyclohexanol acetate; Dorisyl; Madeflor; NSC 163103; Oryclone, Oryclone special, Oryclon extra; p-t-BCHA; p-tert-Butylcyclohexyl acetate; para-tert-Butylcyclohexyl acetate; PTBCHA; Velvetone; Verbeniax; Vertenex; Vertinate; Vertopol; Ylanate	

Current regulation: /

Clinical data:
In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% “Vertenex ®” in pet., tested in 107 consecutive patients in High Wycombe, were observed (15).

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (59).

p-tert -Butyldihydrocinnamaldehyde	
CAS # ***18127-01-0***	
EC # 242-016-2	

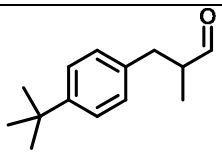
4-(1,1-Dimethylethyl)-benzenepropanal	
p-tert-Butyl-hydrocinnamaldehyde; 3-(4-tert-Butylphenyl)propanal; Bourgeonal; p-tert-Butyldihydrocinnamaldehyde	

Current regulation: III/155

Clinical data: /

Additional information:
It is a “top 200” substance and classified as R43 (IFRA, pers. comm.2010)

http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=39132

BUTYLPHENYL METHYLPROPIONAL (Lilial®)	
CAS # 80-54-6	
EC # 201-289-8	
3-(4-tert-Butylphenyl)-2-methylpropanal	
p-t-Butyl-alpha-methylhydrocinnamic aldehyde; 2-(4-tert-Butylbenzyl)propionaldehyde (REACH, EINECS); 4-(1,1-Dimethylethyl)-α-methyl-benzenepropanal; Hydrocinnamaldehyde, p-tert-Butyl-α-methyl-; (±)-2-Methyl-3-(4-tert-butylphenyl)propanal; 2-Methyl-3-(4-tert-butylphenyl)propanal; 2-[(4-tert-Butylphenyl)methyl]propanal; 3-(4-tert-Butylphenyl)-2-methylpropanal; 3-(p-tert-Butylphenyl)-2-methylpropionaldehyde; 3-(p-tert-Butylphenyl)isobutylaldehyde; 4-(1,1-Dimethylethyl)-α-methylbenzenepropanal; 4-tert-Butyl-α-methylhydrocinnamic aldehyde; Lilestralis; Lilial; Lysmeral; NSC 22275; lilestral; p-tert-Butyl-α-methylhydrocinnamaldehyde; p-tert-Butyl-α-methylhydrocinnamic aldehyde; pt-Bucinal; α-Methyl-p-tert-butylhydrocinnamaldehyde; β-Lilial	

Current regulation: Annex III, part 1, n° 83

Clinical data:

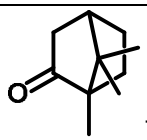
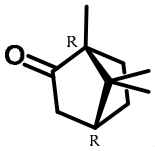
In the “background information” section of the 1999 opinion, lilial is classified as “less frequently reported allergen”; with 2 cases of contact allergy reported in 1 study of 176 eczema patients and 1 case with contact allergy to Lilial from a deodorant; a number of other reported positive cases were considered to possibly have been false positive (33).

Since the last SCCNFP-opinion of 1999, the Frosch 2002a study yielded 0.2% positive reactions to Lilial® (10% pet.) among the 1855 consecutive patients tested (16). The IVDK 2007 study yielded 0.4% (95% CI: 0.2 – 0.8%) positive reactions in 2004 patients consecutively tested (4). The IVDK 2010 study, 0.62% (95% CI: 0.04 – 1.21%; percentages standardised for age and sex) of 1947 patients PTed reacted to the compound (7). In the Groningen 2009 study, n=2, i.e. 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen, tested at only 1% pet. (6). In the deGroot 2000 study, 9 of 1825 consecutively tested patients had a positive reaction to lilial® (5%

pet.) (12). Lilial® has been identified as constituent of perfumes used by a patient, causing ACD (60).

Additional information:

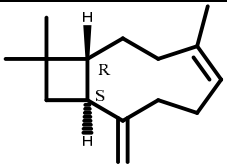
It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010).

CAMPHOR	 76-22-2  464-49-3
CAS # 76-22-2 / 464-49-3	
EC # 207-355-2 / 200-945-0	
1,7,7-Trimethyl-bicyclo[2.2.1]heptan-2-one (76-22-2) **(1R,4R)-1,7,7-Trimethyl-bicyclo[2.2.1]heptan-2-one** (464-49-3)	
76-22-2: DL-Bornan-2-one (REACH, EINECS); 2-Bornanone; Bornan-2-one, INCI name according to CAS; CAMPHOR/DL-bornan-2-one; Camphor; (±)-Camphor; DL-Camphor; 1,7,7-Trimethylnorcamphor; 2-Camphanone; Alphanon; Borneo camphor; Root bark oil; Spirit of camphor **464-49-3**: (1R)-1,7,7-Trimethyl-bicyclo[2.2.1]heptan-2-one; (1R,4R)-(+)- Camphor; (+)-2-Bornanone; (+)-Camphor; (1R)-(+)-Camphor; (1R)-Camphor; (1R,4R)-(+)-Camphor; (R)-(+)-Camphor; (R)-Camphor; Camphor; D-Camphor; D-(+)-Camphor; Alcanfor; Japanese camphor.	

Current regulation: /

Clinical data:
From the UK, a case of allergic contact dermatitis after application of Earex ® ear drops due to rectified camphor oil (tested 10% pet.) was reported (61). Application of a liquid rubefacient of Asian origin caused allergic contact dermatitis in a 58-year-old patient, according to the positive PT result with 10% camphor (“alcaonfor”) in pet. due to this ingredient (62). In the US, a case of contact dermatitis due to “Vics VapoRub” has been reported (63).

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

beta-CARYOPHYLLENE	
CAS # 87-44-5	
EC # 201-746-1	
(1R,4E,9S)-4,11,11-Trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene	
(E)-(1R,9S)-(-)-4,11,11-Trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene; [1R-(1R*,4E,9S*)]-4,11,11-Trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene; (-)-(E)-Caryophyllene; (-)-Caryophyllene; (-)-E-Caryophyllene; (-)-trans-Caryophyllene; (-)-β-Caryophyllene; (E)-Caryophyllene; Caryophyllene; Caryophyllene B; NSC 11906; l-Caryophyllene; trans-Caryophyllene; β-Caryophyllen; β-Caryophyllene; (-)-β-Caryophyllene	

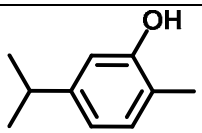
Current regulation: /

Clinical data:

In the Frosch 2002 b study, 0.6% positive reactions to caryophyllene (5% pet.) in 1606 consecutive were observed (17).

Additional information:

beta-Caryophyllene autoxidizes at air exposure. As the primary oxidation products, the hydroperoxides, are very unstable and immediately form epoxides with low sensitizing capciaty, the increase in allergenic activity caused by autoxidation is comparably low (64). A multicenter study identified 0.5% positive reactions to oxidized *beta*-caryophyllene (3.0% pet.) in 1511 consecutive patients (65). Of these, 2 patients (0.1%) reacted to the major oxidation product (caryophyllene oxide) (3.9% pet.).

CARVACROL	
CAS # 499-75-2	
EC # 207-889-6	
2-Methyl-5-(1-methylethyl)-phenol	
2-Hydroxy-1-methyl-4-(1-methylethyl)benzene; 2-Hydroxy-p-cymene; 2-Methyl-5-(1-methylethyl)phenol; 2-Methyl-5-isopropylphenol; 3-Isopropyl-6-methylphenol; 5-Isopropyl-2-methylphenol; 5-Isopropyl-o-cresol; 6-Methyl-3-isopropylphenol; Antioxine; Dentol; Isopropyl o-cresol; Isothymol; NSC 6188; p-Cymen-2-ol	

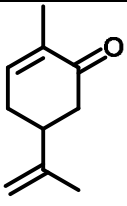
Current regulation: /

Clinical data:

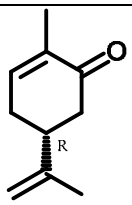
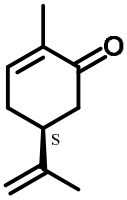
The DeGroot 1985 study identified 2 (1.1%) positive reactions among 179 patients using a 5% PT preparation of this compound – these reactions may have been at least partly due to an “excited back syndrome” and are thus of limited evidence (25). Meynadier et al. [11] patch tested 28 patients with contact allergy to fragrance ingredients using 2% carvacrol in pet. Positive reactions were observed in 3 of 28 patients (after (66)).

Additional information:

Carvacrol is derived from p-cymene by sulfonation followed by alkali fusion. Carvacrol can also be derived from savory, thyme, marjoram, oregano, lovage root, and Spanish origanum oil (66). Carvacrol is a flavor ingredient that can be found in alcoholic beverages, baked goods, chewing gum, condiment relish, frozen dairy, gelatin pudding, non-alcoholic beverages, and soft candy at concentrations from 0.1 to 28.54 ppm (RIFM 2001, according to (66).

CARVONE	 99-49-0
CAS # 99-49-0 / 6485-40-1 / 2244-16-8	
EC # 202-759-5 / 229-352-5 / 218-827-2	
2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-one (99-49-0)	

[11] Meynadier, J. M., J. Meynadier, J. L. Peyron, and L. Peyron. 1986. Clinical forms of skin manifestations in allergy to perfume. *Ann. Dermatol. Venerol.* 113:31–39.

(5R)-2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-one (6485-40-1) **(5S)-2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-one** (2244-16-8)	 6485-40-1  2244-16-8
99-49-0: p-Mentha-6,8-dien-2-one; (±)-Carvone; 2-Methyl-5-isopropenyl-2-cyclohexenone; 5-Isopropyl-2-methyl-2-cyclohexen-1-one; Carvone; DL-Carvone; Karvon; Limonen-6-one; NSC 6275; p-Mentha-1(6),8-dien-2-one **6485-40-1**: R)-(-)-p-Mentha-6,8-dien-2-on); (-)-(5R)-Carvone; (-)-(R)-Carvone; (-)-Carvone; (-)-p-Mentha-6,8-dien-2-one; (4R)-(-)-Carvone; (R)-(-)-Carvone; (R)-Carvone; L-(-)-Carvone; L-Carvone; l-1-Methyl-4-isopropenyl-6-cyclohexen-2-one; l-Carvone **2244-16-8**: (S)-(+)-p-Mentha-6,8-dien-2-one; (+)-Carvone; (S)-(+)-Carvone; (S)-(+)-p-Mentha-6,8-dien-2-one; (S)-Carvone; (+)-Carvone; D-(+)-Carvone; D-Carvone; Talent; d-1-Methyl-4-isopropenyl-6-cyclohexen-2-one; (S)-2-Methyl-5-(1-methylvinyl)cyclohex-2-en-1-one; d-Carvone	

Current regulation: /

Clinical data:

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

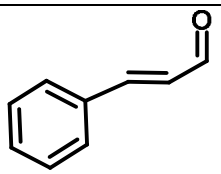
“Carvone has occasionally been reported as an allergen, usually in flavourings. Isomers of carvone have been either a mint or a rye flavour and aroma. We report a woman with positive patch-test reactions to carvone (newly added to the North American Contact Dermatitis Group standard series) and dermatitis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

Additional information:

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

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

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

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

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

Clinical data:

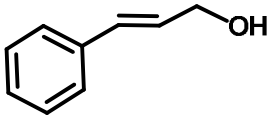
In the “background information” section of the previous opinion (33), cinnamal, one of the 8 constituents of the FM I, is classified as frequent allergen, causing allergic reactions in a notable persons with eczema from cosmetic products in several studies (33).

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

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

Additional information:

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

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

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

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

Clinical data:

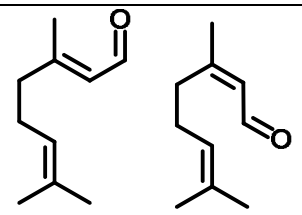
In the "background information" section of the previous opinion (33), cinnamyl alcohol, one of the 8 constituents of the FM I, is classified as frequent allergen, causing allergic reactions in a notable persons with eczema from cosmetic products (33).

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

Additional information:

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

A RIFM review is available (81)

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

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

Clinical data:

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

cosmetic products (33).

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

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

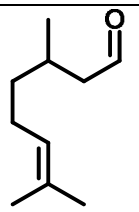
Additional information:

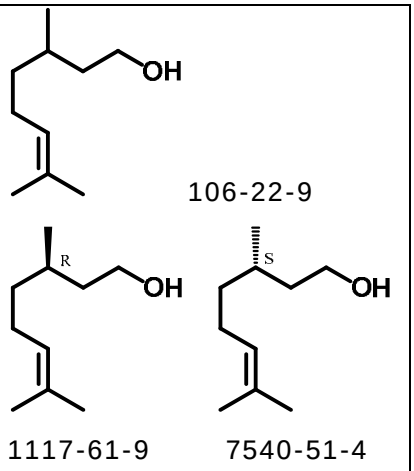
Citral is the mixture of two isomers: cis-citral (neral) and trans-citral (geranial).

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

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

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

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

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

Clinical data:

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

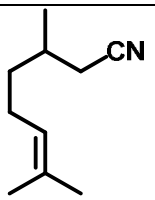
Since the last SCCNFP-opinion of 1999, in the Larsen 2002 c study, „DL citronellol“ (5% in pet.) elicited positive PT reactions in 8.7% of the patients (1). In 1855 consecutive patients of the Frosch 2002 a study, 0.4% positive reactions were noted (16). In the EU 2005 study, 4 of 1701 patients (0.2%,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

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

Additional information:

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

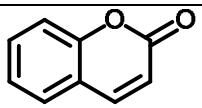
RIFM reviews have been published regarding L-citronellol (86), D-citronellol (87) and DL-citronellol (88). Another review is available by Hostynek and Maibach (89). It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

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

Current regulation: /

Clinical data: /

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

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

Current regulation: Annex III, part1, n° 77

Clinical data:

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

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

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

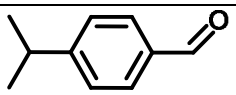
V. Mutterer et al. present the case of a 44 year old patient in whom coumarin was identified as culprit allergen by controlled ROAT testing with 1%, after having caused dermatitis by the use of a deodorant containing coumarin at 0.23% and an EdT (90).

Additional information:

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

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

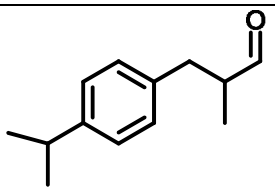
Coumarin is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

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

Current regulation: /

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

Additional information: …

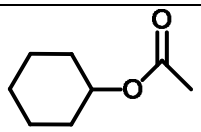
CYCLAMEN ALDEHYDE	
CAS # 103-95-7	
EC # 203-161-7	
α-Methyl-4-(1-methylethyl)-benzenepropanal	
p-Isopropyl-α-methyl-hydrocinnamaldehyde; 2-Methyl-3-(4-isopropylphenyl)propionaldehyde; 2-Methyl-3-(p-isopropylphenyl)propionaldehyde; 3-(4-Isopropylphenyl)-2-methylpropanal; 3-(p-Isopropylphenyl)-2-	

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

Current regulation: …

Clinical data:
/

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

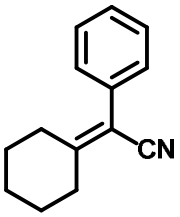
CYCLOHEXYL ACETATE	
CAS # 622-45-7	
EC # 210-736-6	
Cyclohexyletanoat	
Acetic acid cyclohexanyl ester; Acetoxycyclohexane; Cyclohexyl acetate; NSC 8772	

Current regulation: /

Clinical data:

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

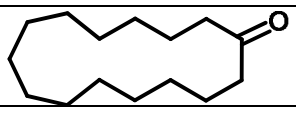
Additional information: A RIFM review is available (92).

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

Current regulation: /

Clinical data: /

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

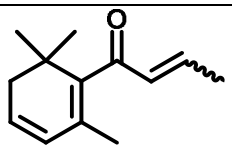
CYCLOPENTADECANONE	
CAS # 502-72-7	

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

Current regulation: /

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

Additional information: …

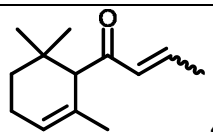
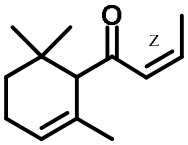
DAMASCENONE ***ROSE KETONE-4*** (Not officially an INCI Name but Perfuming Name; Damascenone as such is not listed in CosIng)	
CAS # 23696-85-7	
EC # 245-833-2	
1-(2,6,6-Trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one	
1-(2,6,6-Trimethyl-1,3-cyclohexadienyl)-2-buten-1-one; 1-Crotonoyl-2,6,6-trimethyl-1,3-cyclohexadiene; 2,6,6-Trimethyl-1-(2-butenoyl)-1,3-cyclohexadiene; 2,6,6-Trimethyl-1-crotonyl-1,3-cyclohexadiene; Rose ketone # 4	

Current regulation: Annex III, part1, n° 160 (max. conc. 0.02%)

Clinical data: /

Additional information:

RIFM reviews are available (93, 94), quoting 1 negative, and 2 positive (2 of 37, 1 of 50 volunteers) HRIPTs with damascenone based on 2 LLNA, the EC3 values were calculated as 1.24% and 1.22%, respectively (94).

alpha-DAMASCONE (TMCHB)	 43052-87-5  23726-94-5
CAS # 43052-87-5 / 23726-94-5	
EC # x / 245-845-8	
1-(2,6,6-Trimethylcyclohex-2-en-1-yl)but-2-enone (43052-87-5); **(2Z)-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one** (23726-94-5)	
43052-87-5: 2,6,6-Trimethyl-1-crotonyl-2-cyclohexene; α-Damascone **23726-94-5**: (Z)-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one; (Z)-α-Damascone; cis-α-Damascone	

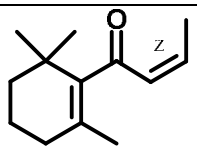
Current regulation: Annex III, part1, n° 157 (max. conc. 0.02%)

Clinical data:
In the Frosch 2002 b study, n=8 (0.5%) mostly strong positive PT reactions to consecutive patients were noted using a mixture of alpha and beta damascene, 0.1% pet. each (17). In human sensitisation experiments, after epicutaneous induction with 30% 1-(2,6,6-trimethylcyclohex-2-en-1-yl)but-2-enone **(**TMCHB, CAS # 43052-87-5) with adjuvant, to enhance response to this weak sensitiser, 8 of 30 patients were elicited by a challenge with 3% TMCHB 2 weeks later (95).

Additional information:

The former CAS # refers to alpha-Damascone or 1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-Buten-1-one. The latter CAS # refers to the identified ingredient cis-alpha-Damascone or (Z)-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one, the content of which is restricted (SCCS-opinion 0392/00).

A RIFM review is available on alpha-damascone (96), quoting a number of partly positive HRIPT and other human studies, as well as different animal experiments. In 1 LLNA reported, an EC3 value of 3.3% was found. Another RIFM review is available for cis-alpha-damascone (97), supplying, however, no data on sensitisation.

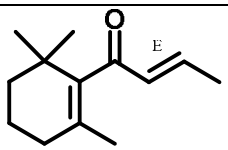
cis-beta-DAMASCONE	
CAS # 23726-92-3	
EC # 245-843-7	
(2Z)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one	
(Z)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one; (Z)-β-Damascone	

Current regulation: Annex III, part 1, n° 162 (max. conc. 0.02%)

Clinical data:
Regarding results of the Frosch 2002 b study, see under alpha-damascone.

Additional information:

A RIFM review is available (98), citing several negative and one positive HRIPTs, and a number of – mostly positive – animal experiments.

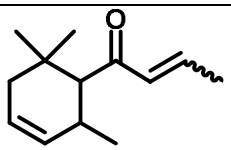
trans-beta-DAMASCONE	
CAS # 23726-91-2	
EC # 245-842-1	
(2E)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one	
(E)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one; (E)-β-Damascone; Damascone beta; trans-2,6,6-Trimethyl-1-crotonylcyclohex-1-ene; trans-β-Damascone; β-Damascone	

Current regulation: Annex III, part 1, n° 158 (max. conc. 0.02%)

Clinical data: /

Additional information:

A RIFM review is available (99), citing 2 negative HRIPT and 1 negative maximisation test, and a number of positive animal experiments (the EC3 value, based on 1 LLNA, was found to be 2.4%).

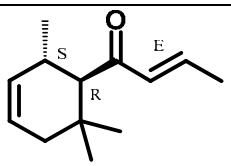
delta-DAMASCONE	
CAS # 57378-68-4	
EC # 260-709-8	
1-(2,6,6-Trimethyl-3-cyclohexen-1-yl)-2-buten-1-one	
δ-Damascone	

Current regulation: Annex III, part 1, n° 161 (max. conc. 0.02%)

Clinical data: /

Additional information:

A RIFM review is available (100), citing several positive HRIPT and 1 negative HRIPT. Cross sensitisation to alpha- and beta-damascone was demonstrated in 3 sensitised subjects. 2 LLNA studies are reported on, yielding EC3 values of 5.19% and 9.6%, resp.

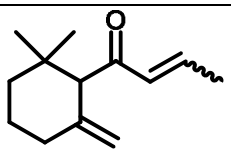
trans-trans-delta-DAMASCONE	
CAS # 71048-82-3	
EC # 275-156-8	
(2E)-rel-1-[(1R,2S)-2,6,6-Trimethyl-3-cyclohexen-1-yl]- 2-buten-1-one	
[1α(E),2β]-1-(2,6,6-Trimethyl-3-cyclohexen-1-yl)-2-buten-1-one; trans-δ-Damascone; δ-Damascone; trans, trans-δ-Damascone	

Current regulation: Annex III, part 1, n° 165 (max. conc. 0.02%)

Clinical data: /

Additional information:

A RIFM review is available (101), citing 1 positive HRIPT (2/15 with 1%).

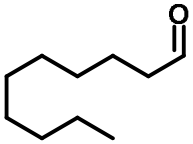
gamma-DAMASCONE	
CAS # 35087-49-1	
EC # 481-910-9	
1-(2,2-Dimethyl-6-methylenecyclohexyl)-2-buten-1-one	
γ-Damascone	

Current regulation: /

Clinical data: /

Additional information:

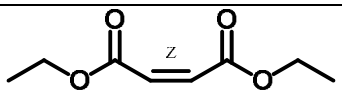
A RIFM review is available (102), citing 1 positive Buehler test and 1 LLNA study yielding an EC3 value of 4.6%

DECANAL	
CAS # 112-31-2	
EC # 203-957-4	
n-Decanal	
Capraldehyde; Capric aldehyde; Caprinaldehyde; Caprinic aldehyde; Decaldehyde; Decanaldehyde; Decyl aldehyde; Decylic aldehyde; NSC 6087; n-Decaldehyde; n-Decyl aldehyde	

Current regulation: /

Clinical data: /

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

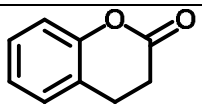
DIETHYL MALEATE	
CAS # 141-05-9	
EC # 205-451-9	
(**2Z)-Diethyl but-2-enedioate**	
2-Butenedioic acid (2Z)-, diethyl ester; 2-Butenedioic acid (Z)-, diethyl ester; Maleic acid, diethyl ester; (2Z)-2-Butenedioic acid diethyl ester; Diethyl (Z)-2-butenedioate; Ethyl maleate; Staflex DEM	

Current regulation: Annex II, n° 426

Clinical data:

In the Malten 1984 study, 3.2% of 182 patients displayed a positive PT reaction to diethyl maleate 0.1% pet. (24). In this study, it has been noted that “in the max. test and clinically this is a strong sensitiser having caused patch test sensitisation (42%)”

Additional information: /

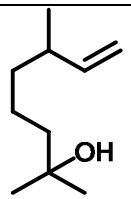
DIHYDROCOUMARIN	
CAS # 119-84-6	
EC # 204-354-9	
3,4-Dihydro-2H-1-benzopyran-2-one	
Hydrocoumarin; Hydrocinnamic acid, o-hydroxy-, δ-lactone; 2-Chromanone; 3,4-Dihydro-1H-benzopyran-2-one; 3,4-Dihydrocoumarin; Dihydrocoumarin; Melilotin; Melilotin (coumarin); Melilotol	

Current regulation: Annex II, n° 427

Clinical data:

In the Malten 1984 study, 3.7% of 182 patients displayed a positive PT reaction to dihydrocoumarine 5% pet. (24).

Additional information: /

DIHYDROMYRCENOL	
CAS # 18479-58-8	
EC # 242-362-4	
(±)-2,6-Dimethyloct-7-en-2-ol	
1,1,5-Trimethyl-6-heptenol; 2,6-Dimethyl-7-octen-2-ol; 3,7-Dimethyl-1-octen-7-ol; 2,6-Dimethyl-7-octen-2-ol (INCI)	

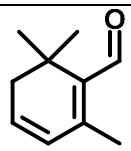
Current regulation: /

Clinical data: /

Additional information:

A RIFM review is available (103), listing 2 negative HRIPTs and 1 negative human maximisation test.

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

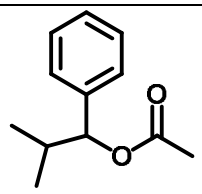
2,3-DIHYDRO-2,2,6-TRIMETHYLBENZALDEHYDE	
CAS # 116-26-7	
EC # 204-133-7	
2,6,6-Trimethyl-1,3-cyclohexadiene-1-carboxaldehyde	
2,2,6-Trimethyl-4,6-cyclohexadien-1-aldehyde; 2,6,6-Trimethyl-1,3-cyclohexadiene-1-aldehyde; Safranal	

Current regulation: /

Clinical data: /

Additional information:

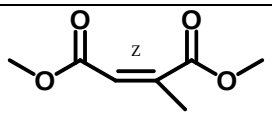
A RIFM review quotes one positive HRIPT (5 of 53) and one negative HRIPT (0 of 54) (93).

DIMETHYLBENZYL CARBINYL ACETATE (DMBCA)	
CAS # 151-05-3	
EC # 205-781-3	
2-Methyl-1-phenylpropyl acetate	
Benzeneethanol, α,α-dimethyl-, acetate; Phenethyl alcohol, α,α-dimethyl-, acetate; 1,1-Dimethyl-2-phenylethyl acetate; 2-Methyl-1-phenyl-2-propyl acetate; 2-Methyl-1-phenylpropan-2-yl acetate; Benzyldimethylcarbinol acetate; Benzyldimethylcarbinyl acetate; Dimethylbenzylcarbinol acetate; Dimethylbenzylcarbonyl acetate; NSC 46123; α,α-Dimethylphenethyl acetate	

Current regulation: /

Clinical data:
In the Frosch 2002 a study, 0.2% positive PT reactions to consecutive patients were noted (16). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and one to 5% DMBCA in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15).

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

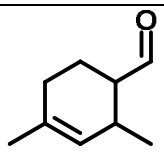
DIMETHYL CITRACONATE	
CAS # 617-54-9	
EC #	
(2Z)-Diethyl-2-metyl-but-2-enedioate	
(2Z)-2-methyl-2-Butenedioic acid, dimethyl ester; 2-Butenedioic acid, 2-methyl-, dimethyl ester, (Z)-; Citraconic acid, dimethyl ester; Dimethyl methylmaleate; Methylmaleic acid, dimethyl ester	

Current regulation: Annex II, n° 431

Clinical data:

In the Malten 1984 study, 3.7% of 182 patients displayed a positive PT reaction to dimethylcitraconate 12% pet. (24). In this paper, a human maximisation test positive in "4/44" is quoted.

Additional information: …

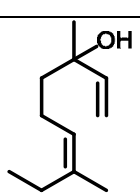
2,4-DIMETHYL-3-CYCLOHEXEN-1-CARBOXALDEHYDE	
CAS # 68039-49-6	
EC # 268-264-1	
2,4-Dimethyl-cyclohex-3-ene-1-carboxaldehyde	
(Z)-Vertocitral C; 2,4-Dimethyl-3-cyclohexene-1-carboxaldehyde; 2,4-Dimethyl-3-cyclohexenecarboxaldehyde; 2,4-Dimethyl-3-cyclohexenylcarbaldehyde; Cyclal C; Ligustral; Tricyclal; Triplal; Tripral; Zestover	

Current regulation: /

Clinical data: /

Additional information:

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

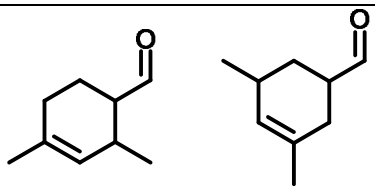
3,7-DIMETHYL-1,6-NONADIEN-3-OL	
CAS # 10339-55-6	
EC # 233-732-6	
(7Z)-3,7-Dimethyl-1,6-nonadien-3-ol	
Ethyl linalool; Methyllinalool	

Current regulation: /

Clinical data: /

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (104), citing 1 negative human maximisation test (n=25).

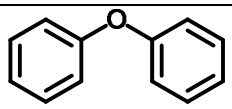
DIMETHYLTETRAHYDRO BENZALDEHYDE	
CAS # 68737-61-1	
EC # 272-113-5	
2,4-Dimethyl-cyclohex-3-ene-1-carboxaldehyde **3,5-Dimethyl-cyclohex-3-ene-1-carboxaldehyde**	2,4- 3,5-
Hivertal; Vertocitral	

Current regulation: /

Clinical data:

In the Larsen 2001 study, 2.3% positive PT reactions were observed with the isomer mixture, tested 5% pet., in 178 patients with known contact allergy to fragrance ingredients (19).

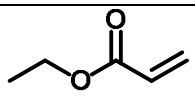
Additional information: /

DIPHENYL ETHER	
CAS # 101-84-8	
EC # 202-981-2	
Phenyl ether	
1,1'-oxybis-Benzene; Barrel Therm 330; Benzene, phenoxy-; Biphenyl oxide; Chemcryl JK-EB; Diphenyl ether; Diphenyl oxide; NSC 19311; Oxybisbenzene; Phenoxybenzene; Phenyl oxide	

Current regulation: /

Clinical data: /

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

ETHYL ACRYLATE	
CAS # 140-88-5	
EC # 205-438-8	
Ethyl 2-propenoate	
Acrylic acid ethyl ester (6CI,8CI); 2-Propenoic acid ethyl ester; Ethyl 2-propenoate; Ethyl acrylate; Ethyl acrylic ester; Ethyl propenoate; NSC 8263	

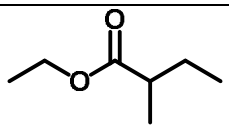
Current regulation: Annex II, n° 435

Clinical data:

In the Malten 1984 study, n=1 (0.5%) of 182 patients displayed a positive PT reaction to ethyl acrylate 1% pet. (24). In the NACDG 2009 multicentre study, 0.9% of

consecutive patients (n=4428) had a positive PT reaction (21).

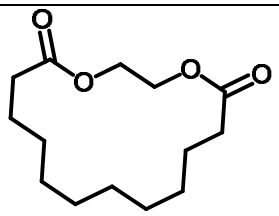
Additional information: /

ETHYL 2-METHYLBUTYRATE	
CAS # 7452-79-1	
EC # 231-225-4	
Ethyl 2-methylbutyrate	
Butyric acid, 2-methyl-, ethyl ester (6CI,7CI,8CI); (±)-Ethyl 2-methylbutanoate; 2-Methylbutanoic acid ethyl ester; 2-Methylbutyric acid ethyl ester; Ethyl 2-methylbutanoate; Ethyl 2-methylbutyrate; Ethyl α-methylbutyrate; NSC 1103	

Current regulation: /

Clinical data: /

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

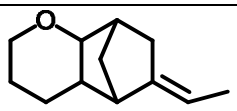
ETHYLENE DODECANEDIOATE	
CAS # 54982-83-1	
EC # 259-423-6	
1,4-Dioxacyclohexadecane-5,16-dione	
Cyclic ethylene dodecanedioate; Ethylene dodecanedioate; Musk 144; Musk C-14	

Current regulation: /

Clinical data:

In the Larsen 2002 c study on 218 patients with known contact allergy to fragrance ingredients, this compound caused 0.9% positive PT reactions at 5% pet. (1).

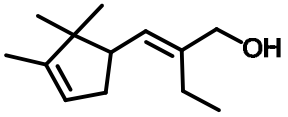
Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

6-ETHYLIDENEOCTAHYDRO-5,8-METHANO-2H-BENZO-1-PYRAN	
CAS # 93939-86-7	
EC # 300-376-9	
6-Ethylideneoctahydro-5,8-methano-2H-1-benzopyran	

Current regulation: /

Clinical data:
In the Larsen 2001 study, no positive PT reactions were observed with this compound, tested 5% pet., in 178 patients with known contact allergy to fragrance ingredients (19).

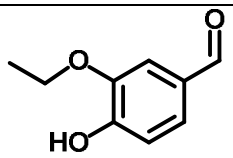
Additional information: /

2-ETHYL-4-(2,2,3-TRIMETHYL-3-CYCLOPENTEN-1-YL)-2-BUTEN-1-OL	
CAS # 28219-61-6	
EC # 248-908-8	
2-Ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)-2-buten-1-ol	
2-Ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)-2-buten-1-ol; 2-Ethyl-4-(2',2',3-trimethylcyclopent-3'-enyl)but-2-enol; Bacdanol; Bangalol; Dartanol; Finanol; Levosandol; Radjanol; Sanjinol	

Current regulation: /

Clinical data: /

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

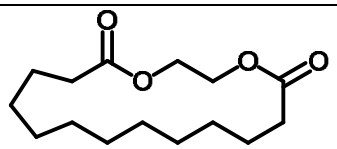
ETHYL VANILLIN	
CAS # 121-32-4	
EC # 204-464-7	
3-Ethoxy-4-hydroxybenzaldehyde	
2-Ethoxy-4-formylphenol; 3-Ethoxy-4-hydroxybenzaldehyde; 3-Ethylvanillin; 4-Hydroxy-3-ethoxybenzaldehyde; Arovanillon; Bourbonal; Ethavan; Ethovan; Ethylprotal; Ethylvanillin; NSC 1803; NSC 67240; Protocatechuic aldehyde ethyl ether; Quantrovanil; Rhodiarome; Vanillal; Vanirom	

Current regulation: /

Clinical data:

The case of a 28-year-old metal grinder with allergic contact dermatitis to a “cutting oil reodorant” has been reported, who tested positively not only to the cutting fluid, the reodorant, but also to several ingredients of the latter product, including “Vanillal S 10026”, 5% pet. (105).

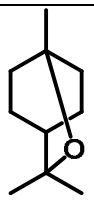
Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

ETHYLENE BRASSYLATE	
CAS # 105-95-3	
EC # 203-347-8	
1,4-Dioxacycloheptadecane-5,17-dione	
Tridecanedioic acid, cyclic ethylene ester; Ethylene glycol, cyclic tridecanedioate; Astratone; Cyclic ethylene glycol tridecanedioate; Cyclic ethylene tridecanedioate; Emeressence 1150; Ethylene brassylate; Musk T; NSC 46155	

Current regulation: /

Clinical data: /

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

EUCALYPTOL	
CAS # 470-82-6	
EC # 207-431-5	
1,3,3-Trimethyl-2-Oxabicyclo[2.2.2]octane	
1,8-Epoxy-p-menthane; 1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octane; 1,8-Cineol; 1,8-Cineole; 1,8-Epoxy-p-menthane; 2-Oxa-1,3,3-trimethylbicyclo[2.2.2]octane; Cajeputol; Cineol; Cineole; Eucalyptol; Eucalyptole; Eucalytol; Eucapur; Eukalyptol; NSC	

6171; Terpan; p-Cineole	

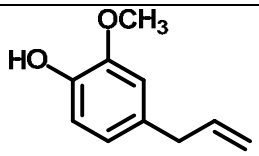
Current regulation: /

Clinical data: /

Additional information:

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

See also ***EUCALYPTUS SPP.*** *LEAF OIL;* eucalyptol is the major ingredient there (up to 85%), but found in significant quantities also in a number of other essential oils (see 3.2).

EUGENOL	
CAS # 97-53-0	
EC # 202-589-1	
2-Methoxy-4-(2-propen-1-yl)-phenol	
Other names: 4-Allyl-2-methoxy-phenol; 1-Allyl-4-hydroxy-3-methoxybenzene; 2-Hydroxy-5-allylanisole; 2-Methoxy-1-hydroxy-4-allylbenzene; 2-Methoxy-4-(2-propenyl)phenol; 2-Methoxy-4-(2'-propenyl)phenol; 2-Methoxy-4-[2-allyl]phenol; 2-Methoxy-4-allylphenol; 3-(3-Methoxy-4-hydroxyphenyl)propene; 3-(4-Hydroxy-3-methoxyphenyl)-1-propene; 4-Allyl-1-hydroxy-2-methoxybenzene; 4-Allyl-2-methoxyphenol; 4-Allylguaiacol; 4-Hydroxy-3-methoxyallylbenzene; Allylguaiacol; Bioxeda; Caryophyllic acid; Dentogum; Eugenic acid; Eugenol; NSC 209525; NSC 8895; p-Allylguaiacol; p-Eugenol	

Current regulation: Annex III, part 1, n° 71

Clinical data:

In the “background information” section of the previous opinion (33), eugenol, one of the 8 components of the FM I, is classified as frequent allergen, causing allergic reactions in about 1.2% in consecutive PT patients and accounting for 4 to 16% of reactions to the FM I. Allergic reactions had been observed in 0.7 – 20% of patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.5% (95% CI: 0.3 – 1.0%) positive reactions in 2065 consecutively PTed patients (4). In the Groningen 2009 study, 1.3% (95% CI: 0.3 – 3.2%) had positive reactions to eugenol, tested at 2% pet., i.e., twice the commonly used concentration (6). F. Giusti et al. examined 1754 consecutive patients tested with eugenol 1% pet. in addition to the baseline series, 09/1998 - 01/2000. 21 patients (1.2%) reacted positively to eugenol (106). In the An 2005 study, 8 of 422 consecutive patients, i.e., 1.9%, had positive reaction (13) (test concentration 2%). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 2.5% positive reactions (22). The IVDK 2010 study, 0.44% (95% CI: 0.04 – 0.84%) of 1214 consecutively tested patients reacted to the compound, while 1.57% (95% CI: 1.19 – 1.95%) of 4801 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=12 reacted positively to eugenol (48).

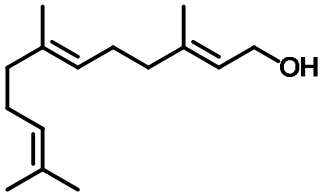
Moreover, eugenol is capable of inducing immediate type reactions of the airways, as illustrated by the well-documented case of a 30 year old hairdresser who developed severe occupational bronchial asthma due to eugenol (107). A case of urticaria after dental treatment with eugenol-containing material was reported from India (108); however, occasional cases are also reported from Europe (109). Occupational exposure to eugenol / zinc oxide type dental restorative material, which is apparently less frequently used nowadays, may lead to occupational sensitisation to eugenol, as illustrated by a case report (110).

Additional information:

Eugenol is the main component (80-95%) of clove oil, but also found in citronella oil, pimento leaf oil and cinnamon bark oil (see section 3.2).

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

FARNESOL



CAS # 4602-84-0

EC # 225-004-1

3,7,11-Trimethyl-2,6,10-Dodecatrien-1-ol

Farnesol; 3,7,11-Trimethyl-2,6,10-dodecen-1-ol; FCI 119a; Farnesyl alcohol; NSC 60597; Nikkosome

Current regulation: Annex III, part 1, n° 82

Clinical data:

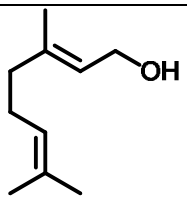
In the "background information" section of the 1999 opinion, farnesol is classified as "less frequently reported allergen"; in 1 study of patients with cosmetic dermatitis 2 cases with contact allergy to farnesol had been reported; in other studies, positive reactions were seen in patients with positive PT reactions to MPR (33).

Since the last SCCNFP-opinion of 1999, farnesol is used not only for its scent, but also for its (slight) antimicrobial activity, useful, for instance, in deodorants. Thus, axillary dermatitis is a relatively typical presentation (111). In a multicentre study based on 1997/98 PT data, 0.5% positive reactions in consecutive patients were noted (Frosch 2002 a (16)). Farnesol is included in the FM II. In the original publication on single constituents of the FM II, 6 of 1701 consecutive patients reacted positively to farnesol 5%, ie., 0.35% (95% CI: 0.13 – 0.77%) (10). In a study on consecutive patients tested in 2003, 38 of 4238 patients had positive reactions to farnesol 5% pet. (0.9%, 95% CI: 0.6 – 1.2%) (4)(IVDK 2007). (A paper on farnesol previously published by the IVDK (112) presents results included in this later analysis.) In a series from Nagoya, Japan, 1.1% positive reactions in 1483 patients with suspected cosmetic dermatitis were observed (tested at 5% pet.) (14). In the Groningen 2009 study, 0.9% (95% CI: 0.2 – 2.7%) had positive reactions (6).

Additional information:

"Farnesol is an acyclic primary sesquiterpene alcohol found in essential oils such as lemongrass, citronella, tuberose blossom, sandalwood and orange blossom" (23). A RIFM review is available (113).

GERANIOL



CAS # 106-24-1

EC # 203-377-1

(2E)-3,7-Dimethyl-2,6-octadien-1-ol

(E)-3,7-Dimethyl-2,6-octadien-1-ol; (E)-Geraniol; (E)-Nerol; 3,7-Dimethyl-trans-2,6-octadien-1-ol; Geraniol; Geranyl alcohol; Lemonol; MosquitoSafe; NSC 9279; trans-3,7-Dimethyl-2,6-octadien-1-ol; trans-Geraniol; β-Geraniol

Current regulation: Annex III, part 1, n° 78

Clinical data:

In the "background information" section of the previous opinion (33), geraniol, one of the 8 components of the FM I, is classified as frequent allergen, causing allergic reactions in about 0.4% in consecutive PT patients and accounting for 3 to 7% of reactions to the FM I. Allergic reactions had been observed in 1.2 – 30% of patients with

eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.5% (95% CI: 0.2 – 0.9%) positive reactions in 2063 consecutively PTed patients (4). In the Groningen 2009 study, 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen, tested at 2%, i.e. twice the usual concentration (6). In a series from Nagoya, Japan, 0.3% positive reactions in 1483 patients with suspected cosmetic dermatitis were observed (tested at the unusually high concentration of 5% pet.) (14). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=7 (0.9%) positive reactions (22). The IVDK 2010 study, 0.39% (95% CI: 0.10 – 0.69%) of 1214 consecutively tested patients reacted to the compound, while 0.87% (95% CI: 0.63 – 1.10%) of 5695 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=17 reacted positively to geraniol (48).

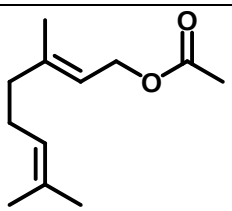
The fact that geraniol also occurs in food flavourings, and can elicit signs and symptoms of manifest contact sensitisation, is illustrated by the case of a 19 year old Japanese woman with cheilitis due to geraniol, improving after avoidance of respective foodstuff (114). A 20 year old Japanese woman with urticaria at the site of application of cosmetics with generalisation (contact urticaria syndrome grade 2), which A. Yamamoto et al. diagnosed as immediate type hypersensitivity to geraniol (without CA) (115).

Additional information:

Geraniol is a component of Palmarosa oil (CYMBOPOGON MARTINI see below), geranium oil (about 40%), citronella oil (30-40%), rose oil, lavender oil, and jasmine oil. It is sensitive to heat which induces autooxidation and isomeric with linalool (53).

Geraniol forms oxidation product with increased sensitizing capacity both via spontaneous autoxidization at air exposure and via metabolic oxidation. Geranial and neral together with hydroperoxide have been identified as oxidation products when geraniol autoxidizes (84). Geranial and neral were also identified as metabolites of geraniol (85). This explains the simultaneous reactions to geraniol and citral seen by (4).

A review is available by Hostynek and Maibach (116) and by RIFM (117). It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010).

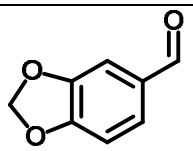
GERANYL ACETATE	
CAS # 105-87-3	
EC # 203-341-5	
(2E)-1-Acetate-3,7-dimethyl-2,6-octadien-1-ol	
(E)-Acetat-3,7-dimethyl-2,6-Octadien-1-ol; Geraniol acetate; (E)-3,7-Dimethyl-2,6-octadien-1-ol acetate; (E)-3,7-Dimethyl-2,6-octadienyl acetate; Acetic acid (2E)-3,7-dimethyl-2,6-octadienyl ester; Acetic acid geraniol ester; Bay pine (oyster) oil; Geranyl acetate; Geranyl ethanoate; NSC 2584; trans-1-Acetoxy-3,7-dimethyl-2,6-octadiene; trans-3,7-Dimethyl-2,6-octadien-1-yl acetate; trans-Geranyl acetate; β-Geranyl acetate	

Current regulation: /

Clinical data: /

Additional information:

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

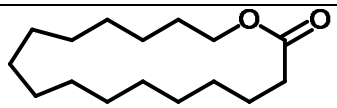
HELIOTROPINE	
CAS # 120-57-0	
EC # 204-409-7	
1,3-Benzodioxole-5-carboxaldehyde	
Piperonal; 2H-Benzo[3,4-d]-1,3-dioxolan-5-ylformaldehyde; 3,4-(Methylenedioxy)benzaldehyde; 3,4-Dihydroxybenzaldehyde methylene ketal; 3,4-Dimethylenedioxybenzaldehyde; 5-Formyl-1,3-benzodioxolane; 5-Formyl-1,3-benzodioxole; 5-Formylbenzodioxole; Benzo[1,3]dioxole-5-carbaldehyde; Benzo[d][1,3]dioxole-5-carboxaldehyde; Geliotropin; Heliotropin; Heliotropine; NSC 26826; Piperonaldehyde; Piperonylaldehyde; Protocatechuic aldehyde methylene ether	

Current regulation: /

Clinical data:

In the Frosch 2002 b study, n=2 (0.2%) positive reactions to “piperonal” (1% pet.) and n=6 (0.4%) to “piperonal” (5% pet.), respectively, in 1606 consecutive were observed (17). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% heliotropine in pet., tested in 106 consecutive patients in Barcelona, were observed (15).

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

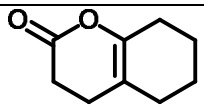
HEXADECANOLACTONE	
CAS # 109-29-5	
EC # 203-662-0	
Oxacycloheptadecan-2-one	
o-Lactone-16-hydroxy-hexadecanoic acid;1,16-Hexadecanolide; 16-Hexadecanolactone; Cyclohexadecanolide; Dihydroambrettolide; Hexadecanoic acid, 16-Hydroxy-, o-lactone; Hexadecanolactone; Hexadecanolide; Juniperic acid lactone; NSC 33546	

Current regulation: /

Clinical data:

In the Larsen 2001 study, 1 of 178 patients with previously diagnosed contact allergy to fragrance ingredients had a positive PT reaction to this compound, tested 5% pet. (19). In the An 2005 study, 6 of 422 consecutive patients, i.e., 1.4%, had positive reactions to 5% “hexadecanolide” (13).

Additional information: /

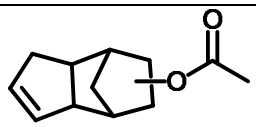
HEXAHYDROCOUMARIN	
CAS # 700-82-3	
EC # 211-851-4	
3,4,5,6,7,8-Hexahydro-2H-1-benzopyran-2-one	
3,4,5,6,7,8-Hexahydro-coumarin; δ-Lactone-2-hydroxy-1-cyclohexene-1-propanoic acid; 3,4,5,6,7,8-Hexahydrocoumarin; Hexahydrocoumarin; Δ-1,6-2-Oxabicyclo(4.4.0)decen-3-one	

Current regulation: Annex II, n° 1135

Clinical data: /

Additional information:

A RIFM review is available (93), p. S115 ff, citing a number of positive human sensitisation experiments.

3a,4,5,6,7,7a-HEXAHYDRO-4,7-METHANO-1H-INDEN-5(OR 6)-YL ACETATE	
CAS # 54830-99-8	
EC # 259-367-2	
3a,4,5,6,7,7a-Hexahydro-4,7-methano-1H-indenol Acetate	
Acetoxydihydrodicyclopentadiene; Cyclacet; Dicyclopentenyl acetate; Dicylat; Tricyclo[5.2.1.02,6]dec-3-enyl acetate; Tricyclodecenyl acetate	

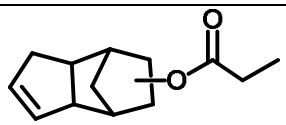
Current regulation: /

Clinical data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 1 to 5% “Cyclacet ®” in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15).

Additional information:

Produced by IFF under the brand name “Cyclacet” (http://www.iff.com/Ingredients.nsf/0/1C9F2CB39EB1EF6480256993002FBC14, last accessed 2010-07-08).

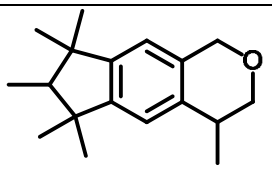
HEXAHYDRO-METHANOINDENYL PROPIONATE	
CAS # 68912-13-0	
EC # 272-805-7	
3a,4,5,6,7,7a-Hexahydro-4,7-methano-1H-indenol propanoate	

3α,4,5,6,7,7α-Hexahydro-4,7-methano-1H-indenyl propionate (Mixture of Isomers); Dicyclopentadiene propionate; tricyclodecenyl propionate; Tricyclo[5.2.1.02,6]dec-3-enyl propionate; Verdyl propionate	

Current regulation: /

Clinical data: /

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

HEXAMETHYLINDANOPYRAN	
CAS # 1222-05-5	
EC # 214-946-9	
1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta[γ]-2-benzopyran	
1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[γ]-2-benzopyrane; 1,3,4,6,7,8-Hexahydro-4,6,6,8,8,8-hexamethylcyclopenta-2-benzopyran; Abbalide; Galaxolide; Galaxolide 50; Galaxolide 50BB; Galaxolide 50IPM; Galaxolide White; HHCB; Pearlide	

Current regulation: /

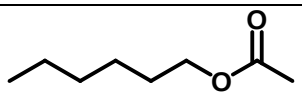
Clinical data:

In the Frosch 2002 a study, n=3 (0.2%) had positive reactions to the compound, tested 10% in isopropyl myristate (with 1 patient reacting positively to the diluent) (16). The Larsen 2001 study, testing with HHCB 7% pet., found 3.4% positive reactions in 178 patients with known contact allergy to fragrance ingredients (19). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had a positive reaction to “Galaxolide 50”, tested at 5% (13) (test concentration 2% pet.). The DeGroot 1985 study identified 3 (1.7%) positive reactions among 179 patients using a 25% PT preparation of HHCB (25). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% “Galaxolide 50 ®” in pet., tested in 100 consecutive patients in Stockholm, were observed (15).

Additional information:

0403/00 - Opinion concerning Hexahydro-hexamethyl-cyclopenta(γ)-2-benzopyran (HHCB)

0610/02 - Opinion on Hexahydro-hexamethyl-Cyclopenta (γ)-2-Benzopyran (HHCB) (no restrictions) It is a “top 100” substance (IFRA, pers. comm.2010).

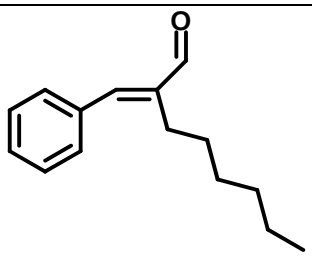
HEXYL ACETATE	
CAS # 142-92-7	
EC # 205-572-7	
Hexyl ethanoate	
Acetic acid, hexyl ester, Hexyl alcohol, acetate; 1-Hexyl acetate; Exceed 600; Hexyl acetate; Hexyl ester acetic	

acid;; NSC 7323; n-Hexyl acetate; n-Hexyl ethanoate	

Current regulation: /

Clinical data: /

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

HEXYL CINNAMAL	
CAS # 101-86-0	
EC # 202-983-3	
α-Hexyl-cinnamaldehyde	
2-(Phenylmethylene)octanal; 2-Hexyl-3-phenyl-2-propenal; 2-Hexylcinnamaldehyde; Hexyl cinnamic aldehyde; NSC 406799; NSC 46150; α-Hexylcinnamaldehyde; α-Hexylcinnamic aldehyde; α-Hexylcinnamyl aldehyde; α-n-Hexyl-β-phenylacrolein; α-n-Hexylcinnamaldehyde	

Current regulation: Annex III, part 1, n° 87

Clinical data:

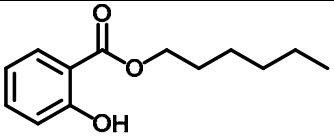
In the “background information” section of the 1999 opinion, hexyl cinnamal (synonymous: alpha-hexyl cinnamal, AHCA) is classified as “less frequently reported allergen”; 2 studies with 1 case and 1 study with 7 cases of contact allergy to this compound in patients with eczema from cosmetic products were found (33).

Since the last SCCNFP-opinion of 1999, in the Frosch 2002 a study, 0.3% positive PT reactions to consecutive patients were noted (16). In the subsequent EU 2005 study, 2 of 1701 patients had positive reactions to AHCA, and n=16 doubtful or irritant to AHCA at 10% in pet. (10). The IVDK 2007 study yielded n=3, i.e, 0.2% (95% CI: 0.03 – 0.4%) positive reactions in 2019 consecutively PTed patients, using 10% pet. as test concentration (4). In the Groningen 2009 study, 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen, using a lower test concentration of 5% pet. (6).

Additional information:

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

Hexyl cinnamal is regarded as “a recommended positive control for skin sensitization testing“, e.g., in the context of the LLNA (118).

HEXYL SALICYLATE	
CAS # 6259-76-3	
EC # 228-408-6	
Hexyl-2-hydroxybenzoate	
Salicylic acid, hexyl ester; 1-Hexyl salicylate; Hexyl salicylate; n-Hexyl salicylate	

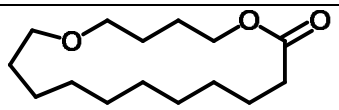
Current regulation: /

Clinical data:

None of the 218 patients with known contact allergy to fragrance ingredients reacted positively to this compound (tested at 5% in pet.) in the Larsen 2002 c study (1).

Additional information:

In a RIFM review, 2 human sensitisation experiments are mentioned which yielded no evidence of sensitising potential (HRIPT, n=103, maximisation test, n=22) (119). It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

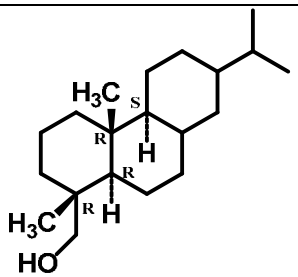
HIBISCOLIDE	
CAS # 6707-60-4	
EC # 229-755-6	
1,6-Dioxacycloheptadecan-7-one	
Undecanoic acid, 11-(4-hydroxybutoxy)-, o-lactone; 12-Oxa-1,16-hexadecanolide; Cervolide; Musk 781; NSC 34741; 12-Oxahexadecan-16-olide	

Current regulation: /

Clinical data:

None of the 178 patients with known contact allergy to fragrance ingredients reacted positively to "12-oxahexadecanolide" (tested at 5% in pet.) in the Larsen 2001 study (19).

Additional information: /

HYDROABIETYL ALCOHOL, when used as a fragrance ingredient	
CAS # 13393-93-6	
EC # 236-476-3	
(1R,4aR,4βS,10aR)-Tetradecahydro-1,4a-dimethyl-7-(1-methylethyl)-1-Phenanthrenemethanol	
Tetradecahydro-1,4a-dimethyl-7-(1-methylethyl)- 1-henanthrenemethanol; Tetrahydroabietyl alcohol	

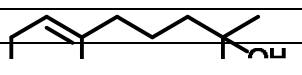
Current regulation: AnnexII, n° 440

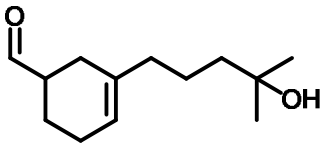
Clinical data:

In the deGroot 2000 study, 17 of 1825 consecutively tested patients had positive reactions to hydroabietyl alcohol (10% pet.) (12).

Additional information:

Commercial hydroabietyl alcohol consists of di- and tetrahydroabitetyl alcohol together with non-modified colophony (120)

HYDROXYISOHEXYL 3-CYCLOHEXENE	

CARBOXALDEHYDE *(HICC) regioisomers*	 51414-25-6
CAS # 31906-04-4 / 51414-25-6	
EC # 250-863-4 / 257-187-9	
4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde (31906-04-4) **3-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde** (51414-25-6)	
31906-04-4: 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexenecarboxaldehyde; 4-(4-Methyl-4-hydroxyamyl)cyclohex-3-ene carboxaldehyde; Lyral	

Current regulation: Annex III, part 1, n° 79

Clinical data:

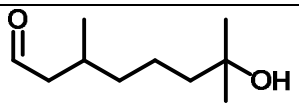
In the “background information” section of the previous opinion (33) HICC is classified as frequent allergen, causing allergic reactions in about 2.8% in consecutive PT patients, two thirds of these being relevant (33).

Since the last SCCNFP-opinion of 1999, in the Frosch 2002 a study, 2.7% of the 1855 consecutive patients reacted positively to HICC (5% pet.) (16). In the EU 2005 study, 28 of 1701 patients (1.7%, 95% CI: 1.1 – 2.4%) reacted positively to 5% HICC in pet. (10). In 21325 patients PTed consecutively in the IVDK 2007 study, 2.4% (95% CI: 2.2 – 2.6%) positive reactions were noted to 5% HICC in pet. (4). Similar to other studies, HICC was the most common single fragrance allergen among 320 patients tested in the Groningen 2009 study, with 3.1% (95% CI: 1.5 – 5.7%) positive reactions despite testing with a lower concentration of 2% pet. (6). In the An 2005 study, 7 of 422 consecutive patients, i.e., 1.7%, had positive reaction (13). The Belsito 2006 study (20) yielded a relatively low prevalence of 0.4% (7 of 1603; exact 95% CI (recalculated): 0.17 – 0.90%) positive reactions with 5% HICC in pet. and even less with lower test concentrations; possible reasons for the much lower prevalence were discussed. The IVDK 2010 study, 2.36% (95% CI: 2.19 - 2.53%) of 37270 consecutively tested patients reacted to HICC (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=8 reacted positively to HICC (48).

Further clinical data with a focus on quantitative dose-response (see also section 4.3), is discussed in (121).

Among the early case reports, S.A. Hendriks reported the case of a 20 year old patient developing axillary dermatitis after 5 months use of a deodorant containing HICC (122).

Additional information: /

HYDROXYCITRONELLAL	
CAS # 107-75-5	
EC # 203-518-7	
7-Hydroxy-3,7-dimethyl-octanal	
(±)-Hydroxycitronellal; 3,7-Dimethyl-7-hydroxyoctanal; 7-Hydroxy-3,7-dimethyloctanal; 7-Hydroxycitronellal; Citronellal hydrate; Citronellal, hydroxy-; Cyclalia; Cyclosia; Cyclosia base; Fixol; Hydroxycitronellal; Laurine; Lilyl aldehyde; Muguet synthetic; Muguettine principle; NSC	

406740; Phixia	

Current regulation: Annex III, part 1, n° 72

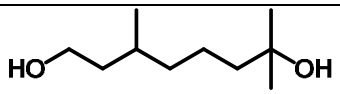
Clinical data:

In the "background information" section of the previous opinion (33), hydroxycitronellal, one of the 8 components of the FM I, is classified as frequent allergen, causing allergic reactions in about 0.75% in consecutive PT patients and accounting for 6 to 16% of reactions to the FM I. Allergic reactions had been observed in 10 – 45% of patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 1.3% (95% CI: 0.9 – 1.9%) positive reactions in 2063 consecutively PTed patients (4). In the Groningen 2009 study, 2.2% (95% CI: 0.9 – 4.5%) had positive reactions to this compound, tested at 2% pet., i.e., twice the commonly used concentration (6). The Sugiura 2000 study observed 1% positive PT reactions (test concentration 5% pet.) in 1483 patients tested for suspected cosmetic dermatitis (14). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 1.5% positive reactions (22). The IVDK 2010 study, 1.17% (95% CI: 0.48 – 1.85%) of 1214 consecutively tested patients reacted to the compound, while 2.95% (95% CI: 2.43 – 3.47%) of 4359 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were tested with hydroxycitronellal, yielding 6 positive reactions (48).

Additional information:

Hydroxycitronellal is a synthetic fragrance, which only recently has been found in a few essential oils, e.g., of a *Narcissus* species and in essential oils of pepper (53)

HYDROXYCITRONELLOL	
CAS # 107-74-4	
EC # 203-517-1	
3,7-Dimethyl-7-octanediol	
2,6-Dimethyl-2,8-octanediol; 3,7-Dimethyl-1,7-octanediol; 3,7-Dimethyloctan-1,7-diol; Citronellol, hydroxy-; Hydroxyciol; Hydroxycitronellol; NSC 406140; NSC 67886	

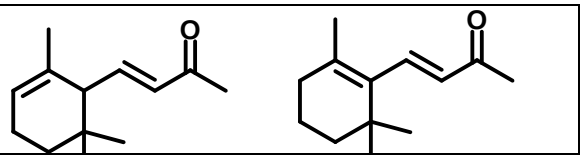
Current regulation: /

Clinical data:

This compound elicited 6.0% positive PT reactions in 218 fragrance sensitive individuals (Larsen 2002 c, (1)).

Additional information:

A RIFM review is available, reporting results of a human induction study (maximisation test) in 25 volunteers, yielding no evidence of sensitisation (123).

IONONE isomeric mixture	
CAS # 8013-90-9	
EC # 232-396-8	

Ionone	
Irisone, mixture of alpha- and beta ionone	

Current regulation: /

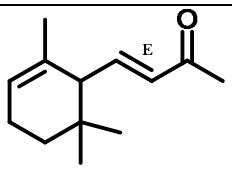
Clinical data: / (see single isomers)

Additional information:

It is a "top 100" substance, further specified with "mixed isomers" (IFRA, pers. comm.2010).

INCI: "MIXED IONONES", with CAS # 14901-07-6 / 6901-97-9 / 8013-90-9 (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=35383, last accessed 2010-07-13).

A RIFM review is available on "ionone" (124), quoting negative human and experimental results.

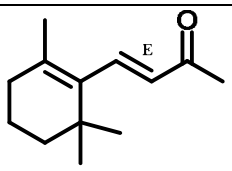
alpha-IONONE	
CAS # 127-41-3	
EC # 204-841-6	
(3E)-4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-Buten-2-one	
(E)-4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-Buten-2-one; (5E)-Ionone; (E)-4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-buten-2-one; (E)-α-Ionone; (±)-trans-α-Ionone; (±)-α-Ionone; 4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-buten-2-one;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

Current regulation: /

Clinical data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% alpha-ionone in pet., tested in 205 consecutive patients, were observed (15).

Additional information: A RIFM review is available (125).

beta-IONONE	
CAS # 79-77-6	
EC # 201-224-3	
(3E)-4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-3-buten-2-one	
(E)-4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-3-buten-2-one; (E)-β-Ionone; Ionone beta; trans-β-Ionone; β-Ionone	

Current regulation: /

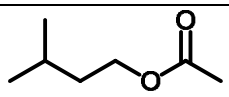
Clinical data:

In the Frosch 1995 dose finding pilot study, no positive reaction to 1% and 5% beta-

ionone in pet., tested in 205 consecutive patients, were observed (15).

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (126).

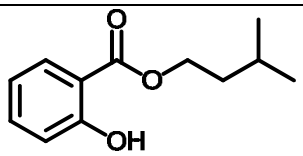
ISOAMYL ACETATE	
CAS # 123-92-2	
EC # 204-662-3	
3-Methylbutyl acetate	
1-Butanol, 3-methyl-, acetate; Acetic acid, isoamyl ester; Isopentyl alcohol, acetate; 3-Methyl-1-butanol acetate; 3-Methyl-1-butyl acetate; 3-Methylbutyl acetate; 3-Methylbutyl ethanoate; Acetic acid 3-methyl-1-butyl ester; Acetic acid 3-methylbutyl ester; Acetic acid isopentyl ester; Banana oil; Isoamyl acetate; Isoamyl alcohol acetate; Isoamyl ethanoate; Isopentyl acetate; Isopentyl ethanoate; NSC 9260; Pear oil; i-Amyl acetate; iso-Amyl acetate; iso-Pentyl acetate	

Current regulation: /

Clinical data: /

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

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

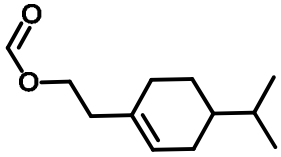
ISOAMYL SALICYLATE	
CAS # 87-20-7	
EC # 201-730-4	
3-Methylbutyl-2hydroxybenzoate	
Isopentyl 2-Hydroxybenzoate; Isopentyl salicylate; Salicylic acid, isopentyl ester (6CI,8CI); Isopentyl alcohol, salicylate; 3-Methylbutyl salicylate; Isoamyl o-hydroxybenzoate; Isoamyl salicylate; Isopentyl salicylate; NSC 7952	

Current regulation: /

Clinical data:

The DeGroot 1985 study identified 1 (0.6%) positive reactions among 179 patients using a 50% PT preparation of this compound – this reaction may have been due to an "excited back syndrome" and is thus a limited evidence (25). In the Frosch 1995 dose finding pilot study, no positive reaction to 1% and 5% isoamyl salicylate in pet., tested in 95 consecutive patients, were observed (15).

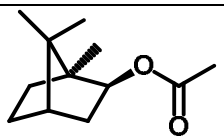
Additional information: A RIFM review is available (127).

ISOBERGAMATE	
CAS # 68683-20-5	
EC # 272-066-0	
4-(Isopropyl)cyclohexadiene-1-ethyl formate	
Structure is incompletly defined 4-(1-Methylethyl)-1,?-cyclohexadiene-1-ethyl formate 4-(Isopropyl)cyclohexadiene-1-ethyl methanoate; menthadienyl formate; Menthadiene-7-methyl formate	

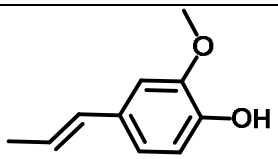
Current regulation: Annex III, part 1, n° 170

Clinical data: /

Additional information: A RIFM review is available (128).

ISOBORNYL ACETATE	
CAS # 125-12-2	
EC # 204-727-6	
(1R,2R,4R)-1,7,7-trimethyl-Bicyclo[2.2.1]hept-2-yl acetate	
Bicyclo[2.2.1]heptan-2-ol, 1,7,7-Trimethyl-, acetate, (1R,2R,4R)-rel- ; Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, exo-; Isoborneol, acetate; (±)-Isobornyl acetate; Isobornyl acetate; NSC 62486; Pichtosin; Pichtosine; exo-Bornyl acetate	

Current regulation: /
Clinical data: In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% isobornyl acetate in pet., tested in 107 consecutive patients in High Wycombe, were observed (15). Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

ISOEUGENOL	
CAS # 97-54-1	
EC # 202-590-7	
2-Methoxy-4-(1-propen-1-yl)-phenol	
Phenol, 2-methoxy-4-(1-propenyl)- ; Phenol, 2-methoxy-4-propenyl-; 1-(3-Methoxy-4-hydroxyphenyl)-1-propene; 2-Methoxy-4-(1-propenyl)phenol; 2-Methoxy-4-propenylphenol; 3-Methoxy-4-hydroxy-1-propenylbenzene; 4-Hydroxy-3-methoxy-1-propenylbenzene; 4-Hydroxy-3-methoxy-β-methylstyrene; 4-Propenylguaiacol; Isoeugenol; NSC 6769	

Current regulation: Annex III, part 1, n° 73

Clinical data:

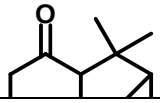
In the “background information” section of the previous opinion (33), isoeugenol, one of the 8 components of the FM I, is classified as frequent allergen, causing allergic reactions in about 1.9% in consecutive PT patients and accounting for 6 to 22% of reactions to the FM I. Allergic reactions had been observed in 2 – 25% of patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 1.3% (95% CI: 0.8 – 1.8%) positive reactions in 2063 consecutively PTed patients (4). In the Groningen 2009 study, 1.3% (95% CI: 0.3 – 3.2%) had positive reactions to isoeugenol, tested at 2% pet., i.e., twice the commonly used concentration (6). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 5.4% positive reactions (22). At St Johns Institute of Dermatology in London 3636 subjects were patch tested with isoeugenol 2001-2005, 97 of whom were positive. Year-on-year incidence showed an increasing trend, with an overall incidence of 2.67% (129). The IVDK 2010 study, 1.62% (95% CI: 0.87 – 2.38%) of 1214 consecutively tested patients reacted to the compound, while 3.41% (95% CI: 2.90 – 3.92%) of 5747 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=11 reacted positively to isoeugenol (48).

Additional information:

Isoeugenol occurs in a cis- (CAS 5912-86-7) and a trans-isomers (CAS 5932-68-3), the latter dominating in trade products (82-88%) (53).

Isoeugenyl methyl ether caused 7.3% positive reactions in the Larsen 2002 c study (1). A number of derivatives of isoeugenol, such as isoeugenyl acetate, transisoeugenol, isoeugenyl benzoate, isoeugenyl phenylacetate, isoeugenyl methyl ether and benzyl isoeugenyl have been examined in 2261 consecutive patients; a varying proportion of positive patch test reactions and a varying proportion of concomitant reactions with isoeugenol have been observed (130). In an earlier study, 5 of 7 patients positive to isoeugenol also displayed positive reactions to isoeugenol acetate (1.2% eth.) (131) (see also section 5 and 6).

ISOLONGIFOLENEKETONE	
CAS # 33407-62-4	

EC # 245-890-3	
1,3,4,6,7,8a-Hexahydro-1,1,5,5-tetramethyl-2H-2,4a-methanonaphthalen-8(5H)-one	
Hexahydro-1,1,5,5-tetramethyl-2H-2,4a-methanonaphthalen-8(5H)-one	

Current regulation: /

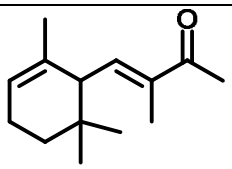
Clinical data:

The Larsen 2001 study idenfied 1 in 178 patients with known contact allergy to fragrance ingredients who reacted positively in the PT (5% pet.) (19).

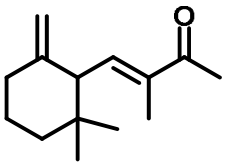
Additional information:

Not listed in CosIng under this CAS #. Other CAS # reported in RIFM review [13]:

- 29461-14-1 CosIng: INCI name "ISOLONGIFOLENE KETONE EXO";
- 23787-90-8 CosIng: INCI name "ISOLONGIFOLANONE";
- 29461-13-0: CosIng: INCI name "HEXAHYDRO-TETRAMETHYLMETHANONAPHTHALEN-8-ONE".

alpha-ISOMETHYL IONONE	
CAS 127-51-5	
EC 204-846-3	
3-Methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	
4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-methyl-3-buten-2-one; Cetone Alpha; Isomethyl-α-ionone; NSC 66432; α-Cetone	

Current regulation: Annex III, part 1, n° 90

gamma-Methylionone	
CAS 7388-22-9	
EC /	

According to CosIng, "alpha-ISOMETHYL IONONE" (CAS # 127-51-5) and "gamma-Methylionone" (CAS # 7388-22-99) are synonyms, with one CAS number, and one preferred chemical name. The substance(s) are accordingly treated in the 1999 opinion (33) as one. As this treatment is also found in the literature, both substances are reviewed together.

[13] Opdyke, D. L. J.; Letizia, C. **Monographs on fragrance raw materials. Isolongifolanone.** Food and Chemical Toxicology (1983), 21(6), 859

Clinical data:

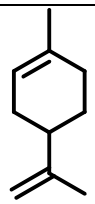
In the “background information” section of the 1999 opinion, “gamma-methylionone” is classified as “less frequently reported allergen”; 1 study with 2 cases and 2 studies with 1 case were found among patients with eczema from cosmetic products (33).

The IVDK 2007 study yielded n=1, i.e, 0.1% (95% CI: 0.00 – 0.2%) positive reactions in 2004 consecutively PTed patients (4). In the subsequent period (2005-2008), n=986 patients were tested in the IVDK 2010 study, with no positive reactions (7). In the Groningen 2009 study, n=2, i.e. 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen, tested at only 1% pet. (6).In a Korean study with 422 consecutive patients, 2.1% reacted positively to “alpha isomethyl ionone (gamma-methylionone), CAS # 127-51-5”, tested 5% pet. (13)

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010) under the label of “alpha-ISOMETHYL IONONE (CAS # 127-51-5)”.

A RIFM review is available, listing 4 human sensitisation experiments employing different study protocols – all yielding negative results (132). Another review is available by Hostynek and Maibach (133), both referring to “alpha-ISOMETHYL IONONE (CAS # 127-51-5)”.

(DL)-LIMONENE	
CAS # 138-86-3	
EC # 231-732-0	
1-Methyl-4-(1-methylethenyl)-cyclohexene	
p-Mentha-1,8-diene; (±)-Dipentene; (±)-Limonene; (±)-α-Limonene; 1,8-p-Menthadiene; 1-Methyl-4-(1-methylethenyl)cyclohexene; 1-Methyl-4-isopropenyl-1-cyclohexene; 1-Methyl-4-isopropenylcyclohexene; 1-Methyl-p-isopropenyl-1-cyclohexene; 4-Isopropenyl-1-methyl-1-cyclohexene; 4-Isopropenyl-1-methylcyclohexene; Cajeputen; Cajeputene; Cinen; Cinene; DL-Limonene; Dipenten; Dipentene; Eulimen; Flavor orange; Goldflush II; Kautschin; Limonen; Limonene; NSC 21446; NSC 844; Nesol; Orange X; Orange flavor; PC 560; Roti-Histol; SF 001; dl-Limonene; α-Limonene	

Current regulation: Annex III, part1, n° 88, 167, 168

Clinical data:

In the “background information” section of the 1999 opinion, d-limonene (CAS 5989-27-5) is classified as “less frequently reported allergen in relation to cosmetic exposure”; with contact allergy to oxidised limonene not infrequently reported in the literature (33).

Since 1999, several studies have been performed using limonene where the oxidation state is not given, but intended to be low. In one study, 0.6% positive reactions to limonene (3% pet.) were observed in 1606 consecutive patients (17). The IVDK 2007 study yielded n=3, i.e. 0.1% (95% CI: 0.03 – 0.4%) positive reactions in 2396 patients consecutively PTed with limonene (2% pet.) (4). The IVDK 2010 study, 0.28% (95% CI: 0 – 0.57%; percentages standardised for age and sex) of 1241 patients PTed with dipentene reacted to the compound (7). In the Groningen 2009 study, no positive reactions to this allergen, tested at 2% pet., were observed in 320 patients (6).

Regarding selected case reports, a case of a 40 year old citrus fruit picker with work related hand dermatitis and bronchial asthma has been described, who tested extreme positive to DL-limonene (2% pet.), and, less extremely, to citronellol and to the biocide dichlorophene (134). Moreover, limonene is used as a solvent in technical applications and cleaning and can lead to allergic contact dermatitis (e.g., a histopathology technicians (135, 136) or a painter and decorator (137)). In “water-free” hand cleansers it is reported to be used in concentrations around 10 – 20% (137). Wax polishes may contain dipentene and have caused one reported case of occupational ACD in a car mechanic (138). Another case of occupational ACD from dipentene in honing oil has been reported (139). In a case series from Sweden, 2 of 105 car mechanics patch tested for occupational contact dermatitis had positive reactions to oxidised *d*-limonene (5% pet.) (140).

Additional information:

Limonene is a monocyclic monoterpene existing in two enantiomers: (R)-(+)-limonene (CAS 5989-27-5) and (S)-(–)-limonene (CAS 5989-54-8). Racemic limonene is known as dipentene.

The allergenicity of limonene is closely related to oxidation (71, 72, 141, 142). It has been demonstrated that both enantiomers, R-(+)- and S-(-)-limonene sponateously autoxidize, and that the primary oxidation products formed, the hydroperoxides, are strong and clinically relevant contact allergens. Among 2411 consecutive patients in a multi-centre European study, 63 (2.6 % [95%CI: 2.0-3.3]) reacted to oxidised (R)-(+)-and/or (S)-(–)-limonene (3.0% pet.) (72). In other multi-studies also, a considerable proportion of patients showed

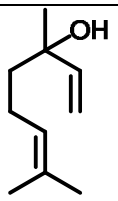
positive patch test reactions to oxidised R-(+)- limonene, e.g.,

- between 0.3% and 5.1% of subgroups of 2800 patients in Stockholm and Leuven, depending on test concentration, oxidation state and department(141),
- between 0.3% and 6.5% in 4 different departments in altogether 2273 patients (72, 143).

The primary oxidation products are the major allergens forming specific antigens (Bråred-Christensson J, Matura M, Bäcktorp C, Börje A, Nilsson JLG, Karlberg A-T. Hydroperoxides form specific antigens in contact allergy. Contact Dermatitis 2006: 55: 230-237.).

Current IFRA standards emphasise "a peroxide value of less than 20 millimoles peroxides per litre, determined according to the FMA method" (http://www.ifraorg.org/Home/Code,+Standards+Compliance/IFRA+Standards/page.aspx/56, last accessed 2009-11-11). For a more general discussion see section 5.

There is no scientific rational for the difference in peroxide value allowed for limonene (20 millimoles peroxides per litre) compared to linalool (10 millimoles peroxides per litre). Specific values for hydroperoxides, which are allergens, would be desirable.

LINALOOL	
CAS # 78-70-6 (isomeric mixture)	
EC # 201-134-4; 245-083-6 See: http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=27933	
3,7-Dimethyl-1,6-octadien-3-ol	
(±)-Linalool; 2,6-Dimethyl-2,7-octadien-6-ol; 2-Methyl-1-prenyl-3-buten-2-ol; 3,7-Dimethyl-1,6-octadiene-3-ol; 3,7-Dimethyl-3-hydroxy-1,6-octadiene; L 260-2; Linalol; Linalool; Linalyl alcohol; Linanool; NSC 3789; dl-Linalool; β-Linalool	

Current regulation: Annex III, part 1, n° 84

Clinical data:

In the "background information" section of the 1999 opinion, linalool in non-oxidized form is classified as "less frequently reported allergen"; with 4 cases of contact allergy reported in 2 studies on patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, studies have been performed on contact allergy to linalool, oxidation state not given, but intended to be low. In the Larsen 2002 c study, none of the 218 patients with known contact allergy to fragrance ingredients had a positive reaction to linalool 5% pet., as prepared specially for this study (1). The IVDK 2007 study yielded 0.3% (95% CI: 0.1 – 0.6%) positive reactions in 2401 patients consecutively tested with stabilised linalool (10% pet.) (4). The IVDK 2010 study, 1 patient had a weak, and another a ++ reaction among the n=985 patients tested with 10% linalool (stabilised) in pet. (7). In the Groningen 2009 study, n=2, i.e. 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen (6). The deGroot 2000 study with 1825 consecutively tested patients yielded 3 positive reactions to linalool (12). The DeGroot 1985 study found no positive reactions among 179 patients using a 30 % PT preparation of linalool (25).

Additional information:

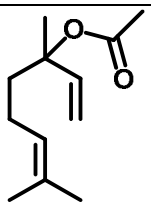
The allergenicity of linalool is closely related to oxidation and the primary oxidation products, the hydroperoxides, are the main allergens (144). In a clinical study 2002-

2003 in 6 European centres including 1511 consecutive patients, 1.3% showed a positive reaction to oxidized linalool (2.0% pet.) and 1.1% to the hydroperoxide fraction (65). A recent dose-response study in Sweden including 3400 patients in two test centres showed a positive reaction in 5.3% of the 1725 patients tested with oxidized linalool 6% pet. (145).

A review by RIFM is available both regarding linalool (146) and linalool "and related esters" (147). Another review is available by Hostynek and Maibach (148).

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

Additional CAS numbers exist for the single isomers: CAS # 126-90-9 (S-isomer), CAS # 126-91-0 (R-isomer); however, in the studies reviewed the isomeric mixture has been used throughout.

LINALYL ACETATE	
CAS # 115-95-7	
EC # 204-116-4	
3,7-Dimethyl-1,6-octadien-3-yl acetat	
1,6-Octadien-3-ol, 3,7-dimethyl-, acetate; Linalool acetate K; (±)-Linaloyl acetate; (±)-Linalyl acetate; 1,5-Dimethyl-1-vinyl-4-hexenyl acetate; 3,7-Dimethyl-1,6-octadien-3-yl acetate; 3-Acetoxy-3,7-dimethyl-1,6-octadiene; Acetic acid linalool ester; Bergamiol; Bergamol; Bergamot mint oil; Linalyl acetate; NSC 2138; dl-Linalool acetate	

Current regulation: /

Clinical data:
In 100 patients tested in Odense, DK, in the early 90s, no positive reactions were observed with 1 and 5% linalyl acetate in pet. (15). In the Frosch 2002 a study, testing with linalyl acetate (10% pet.), 0.2% positive PT reactions to consecutive patients were noted (16). Similarly, the RIFM review mentioned quotes a number of studies where no allergic reactions to this compound had been observed, with the exception of one positive reaction in a Dutch study in 1988(149).

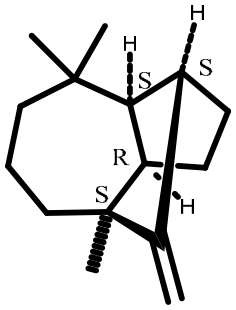
Additional information:

This is the main component of lavender oil (30%), also part of bergamot oil, neroli oil, peppermint oil, lemon oil and jasmine oil (53).

Linalyl acetate autoxidizes spontaneously at air exposure and the major allergens, the hydoperoxides, are the primary oxidation products (150). The pattern of autoxidation is similar to that for linalool and as the acetate can be be metabolically hydrolysed to the corresponding alcohol cross reactions to allergens from oxidized linalool should be possible. This was indicated in a study of lavender oil and oxidised linalyl acetate which elicited positive PT reactions in some patients with known contact allergy to oxidised linalool (n=3) (151).

A RIFM review is available reporting 7 human sensitisation experiments yielding few or no cases of sensitisation (152).

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

Longifolene	
CAS # 475-20-7	
EC # 207-491-2	
(1S,3aR,4S,8aS)-Decahydro-4,8,8-trimethyl-9-methylene-1,4-methanoazulene	
1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, (1S,3aR,4S,8aS)-(+)-; 1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, [1S-(1α,3aβ,4α,8aβ)]-; (+)-Longifolene; Junipen; Junipene; Kuromatsuen; Kuromatsuene; Longifolen; NSC 150808; d-Longifolene; α-Longifolene	

Current regulation: /

Clinical data: /

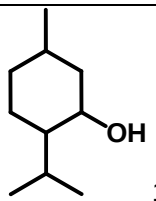
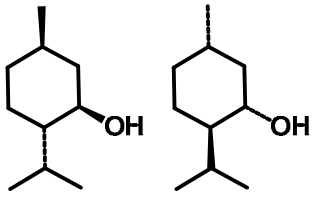
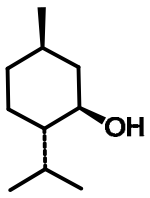
Additional information:

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

http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=77412

This substance is listed in the Register of Flavouring Substances pursuant to Article 3(1) of Regulation EC No. 2232/96 (28 Oct 1996) that lays down a procedure for flavouring substances used or intended for use in or on foodstuffs. Adopted February 23, 1999.

A RIFM review is available citing one negative human maximisation test (n=25) with 10% pet. (153).

MENTHOL	 1490-04-6  89-78-1  2216-51-5
CAS # 1490-04-6 / 89-78-1 / 2216-51-5	
EC # 216-074-4 / 239-388-3 / 218-690-9	
5-Methyl-2-(1-methylethyl)-cyclohexanol (1490-04-6) **(1R,2S,5R)-rel-5-Methyl-2-(1-methylethyl)-cyclohexanol** (89-78-1) **(1R,2S,5R)-5-Methyl-2-(1-methylethyl)-cyclohexanol** (2216-51-5)	
Other names: **1490-04-6**: Menthol; 1-Methyl-4-isopropyl-3-cyclohexanol; 2-Isopropyl-5-methylcyclohexan-1-ol; 2-Isopropyl-5-methylcyclohexanol; 3-Hydroxy-p-menthane; 5-Methyl-2-(1-methylethyl)cyclohexanol; 5-Methyl-2-isopropylcyclohexanol; Menthyl alcohol; p-Menthan-3-ol **89-78-1**: (1α,2β,5α)-5-Methyl-2-(1-methylethyl)-cyclohexanol; cis-1,3,trans-1,4-Menthol; dl-Menthol; (1R,2S,5R)-rel-5-Methyl-2-(1-methylethyl)cyclohexanol; (±)-Menthol; DL-Menthol; Fisherman's Friend Lozenges; Hexahydrothymol; Menthacamphor; Menthol; Menthomenthol; NSC 2603; Peppermint camphor; Racementhol; Therapeutic Mineral Ice; Thymomenthol; rac-Menthol	

2216-51-5: (1R,2S,5R)-5-Methyl-2-(1-methylethyl)-cyclohexanol; [1R-(1α,2β,5α)]-5-Methyl-2-(1-methylethyl)-cyclohexanol; (1R,3R,4S)-(-)-Menthol; (-)-Menthol; (-)-Menthyl alcohol; (-)-trans-p-Methan-cis-3-ol; (1R)-(-)-Menthol; (1R,2S,5R)-(-)-Menthol; (1R,2S,5R)-2-Isopropyl-5-methylcyclohexan-1-ol; (1R,2S,5R)-2-Isopropyl-5-methylcyclohexanol; (R)-(-)-Menthol; 1R-Menthol; L-Menthol; L-Mentholum; Levomenthol; NSC 62788; l-(-)-Menthol; l-Menthol	

Current regulation: /

Clinical data:

Among 512 patients referred from a dental department for diagnostic work-up of various intraoral symptoms and complaints within 4 years, 10 patients had positive (+ to +++) PT reactions to menthol 5% pet. at D4, mostly reporting dramatic improvement after cessation of use of peppermint-containing oral products (154). In 63 patients positive to the FM I, 1 had a positive PT reaction to menthol, 5% pet., in the Santucci 1987 study (28). The IVDK 2010 study, 1 of 1147 patients tested with 1% menthol in pet. had a weak positive reaction to menthol (7).

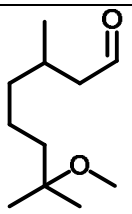
A case of contact allergy to “peppermint and menthol” in a transdermal therapeutic system with flurbiprofen for lumbar pain has been described (155).Moreover, a case of rhinitis caused by different menthol-containing products, diagnostically proven by repeatedly positive urticarial reactions after application of 2% menthol in pet. or 5% peppermint oil in pet., has been reported (156). “A case of asthma due to menthol is reported in a 40-year-old woman with no history of asthma or any other allergy. During the last two years, the patient had presented dyspnoea, wheezing and nasal symptoms when exposed to mentholated products such as toothpaste and candies. The aetiology was suggested by the history of exposure and diagnosis was established by skin tests and bronchial challenge with menthol. The patient achieved control of symptoms by avoiding menthol and its derivatives.“ (157).

Additional information:

Menthol is an ingredient of several essential oils, like peppermint oil, and has been identified as causative allergen in case reports listed above.

Four stereoisomeric forms are known. Natural menthol occurs as L-form (CAS 2216-51-5), trade products are DL-menthol (CAS 1490-04-6). D-form: CAS 89-78-1, racemic: CAS 15356-70-4**.** Sensitive to light, air and heat (53).

L-menthol and menthol (isomer not specified) are “top 100” substances (IFRA, pers. comm.2010). RIFM reviews are available regarding “menthol” (158), D-menthol (159), L-menthol (160), DL-menthol (161) and menthol, racemic (162). A CIR expert panel review is available (163).

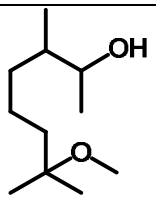
METHOXYCITRONELLAL	
CAS # 3613-30-7	
EC # 222-784-5	
7-Methoxy-3,7-dimethyl-octanal	
7-Methoxy-3,7-dimethyloctanal; 7-Methoxy-6,7-dihydrocitronellal; 7-Methoxycitronellal; Methoxycitronellal; Methoxydihydrocitronellal	

Current regulation: /

Clinical data:

Nakayama et al. found 1974 (after (29)) 12 “strong positive” and 10 “weak positive” reactions to methoxycitronellal (unknown test concentration), with cross-reactions to hydroxycitronellal (proportion not given), in 183 patients.

Additional information: /

METHOXYTRIMETHYLHEPTANOL	
CAS # 41890-92-0	
EC # 255-574-7	
7-Methoxy-3,7-dimethyl-2-octanol	
3,7-Dimethyl-7-methoxy-2-octanol; Dihydromethoxyelgenol; Elesant; Osyrol	

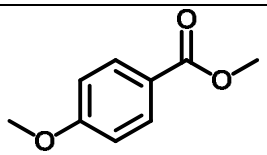
Current regulation: /

Clinical data:

In the Larsen 2002 c study, 0.9% of the patients with known contact allergy to fragrance ingredients had a positive PT reaction to this ingredient not reported as allergen previously (1).

Additional information:

A RIFM review is available (128) citing 1 negative maximisation test (n=27).

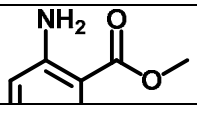
METHYL p-ANISATE	
CAS # 121-98-2	
EC # 204-513-2	
Methyl-4-methoxybenzoate	
p-Anisic acid, methyl ester; 4-(Methoxycarbonyl)anisole; 4-Methoxybenzoic acid methyl ester; Methyl p-anisate; Methyl p-methoxybenzoate; NSC 7324; p-Methoxybenzoic acid methyl ester	

Current regulation: /

Clinical data:

In the Malten 1984 study, n=1 (0.5%) of 182 patients displayed a positive PT reaction to methyl anisate 4% pet. (24).

Additional information: /

METHYL ANTHRANILATE	
CAS # 134-20-3	

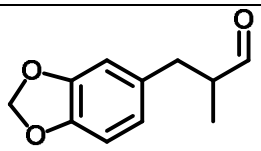
EC # 205-132-4	
Methyl 2-aminobenzoate	
Anthranilic acid, methyl ester; 2-(Methoxycarbonyl)aniline; 2-Aminobenzoic acid methyl ester; 2-Carbomethoxyaniline; Bird Shield; Grain 96-1; Methyl 2-aminobenzoate; Methyl 6-aminobenzoate; Methyl anthranilate; Methyl o-aminobenzoate; NSC 3109; ReJex-iT; Rejex-iT AP 50; Rejex-iT TP 40; Sunarome UVA; [2-(Methoxycarbonyl)phenyl]amine; o-(Methoxycarbonyl)aniline; o-Aminobenzoic acid methyl ester; o-Carbomethoxyaniline	

Current regulation: /

Clinical data:

In 91 Israeli patients with a positive or doubtful reaction to FM I or MP methyl anthranilate was tested (conc. not given), with a negative result (164).

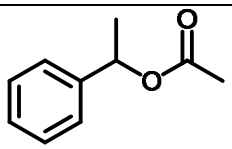
Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

METHYLENEDIOXYPHENYL METHYLPROPANAL	
CAS # 1205-17-0	
EC # 214-881-6	
3-(1,3-Benzodioxol-5-yl)-2-methylpropanal	
Hydrocinnamaldehyde, α-methyl-3,4-(methylenedioxy)-; 2-Methyl-3-(3,4-methylenedioxyphenyl)propanal; 2-Methyl-3-(3,4-methylenedioxyphenyl)propionaldehyde; 3-(3,4-Methylenedioxyphenyl)-2-methylpropanal; Heliobouquet; Heliofresh; Heliogan; Helional; Helipropanal; NSC 22282; Tropional; α-Methyl-1,3-benzodioxole-5-propanal; α-Methyl-3,4-(methylenedioxy)hydrocinnamaldehyde	

Current regulation: /

Clinical data: /

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

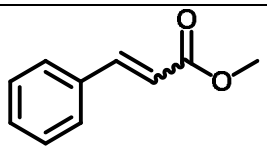
METHYLBENZYL ACETATE	
CAS # 93-92-5	
EC # 202-288-5	
1-Phenylethyl acetate	
Benzenemethanol, α-methyl-, acetate ; Benzyl alcohol, α-methyl-, acetate ; (±)-Styrallyl acetate; (±)-α-Methylbenzyl acetate; (±)-α-Phenethyl acetate; 1-Acetoxy-1-phenylethane; 1-Phenylethyl acetate; Gardeniol II; Gardenol; Methyl phenyl carbinyl acetate; Methylphenylcarbinol acetate; NSC 2397; Styrallyl acetate;	

Styrylallyl acetate; dl-1-Phenylethyl acetate; sec-Phenethyl acetate; sec-Phenylethyl acetate; α-Methylbenzenemethanol acetate; α-Methylbenzyl acetate; α-Methylbenzyl alcohol, acetate; α-Phenethyl acetate; α-Phenylethyl acetate	

Current regulation: /

Clinical data: /

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

METHYL CINNAMATE	
CAS # 103-26-4	
EC # 203-093-8	
Methyl 3-phenylprop-2-enoate	
3-Phenyl-2-propenoic acid methyl ester; Cinnamic acid, methyl ester; 3-Phenyl-2-propenoic acid methyl ester; 3-Phenylacrylic acid methyl ester; Methyl 3-phenyl-2-propenoate; Methyl 3-phenylacrylate; Methyl 3-phenylpropenoate; Methyl cinnamate; Methyl cinnamylate; NSC 9411; SemaSORB 9815	

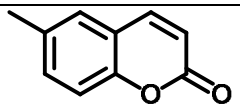
Current regulation: /

Clinical data:

Patch tests with some components of Peru balsam were carried out at 8 worldwide centers in 142 patients who had previously reacted to 25% MP. Reactions to methyl cinnamate (dose and vehicle not reported) were observed in 6 of 142 patients (no further details reported) (165).

Additional information:

A RIFM review is available (166), reviewing, e.g., a number of animal studies with conflicting results. See also under Myroxylon pereirae.

6-METHYL COUMARIN	
CAS # 92-48-8	
EC # 202-158-8	
6-Methylchromen-2-one	
Coumarin, 6-methyl-; 6-MC; 6-Methyl-2H-1-benzopyran-2-one; 6-Methyl-2H-chromen-2-one; 6-Methylbenzopyrone; 6-Methylcoumarin; 6-Methylcoumarinic anhydride; NSC 5870; Toncarine	

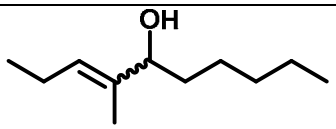
Current regulation: Annex III, part 1, n° 46

Clinical data:

Two of 24 white volunteers developed a photoallergic reaction after single epicutaneous exposure with 5% methyl coumarin in ethanol and UV-A radiation (16 J/cm²). After a photomaximisation test, 6 of 10 subjects developed photocontact allergic reactions

(167). Cardoso et al. report on 2 photoallergic patch test reactions to this substance, which were apparently clinically relevant, in 83 Portugese patients tested (168). Similar results (2 of 76 patients with positive photopatchtest) were reported from New York (169).

Additional information: /

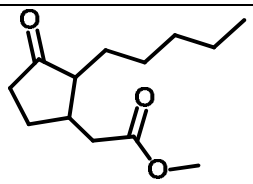
METHYL DECENOL	
CAS # 81782-77-6	
EC # 279-815-0	
4-Methyl-3-decen-5-ol	

Current regulation: /

Clinical data: /

Additional information:

A RIFM review is available (170), reporting 1 negative HRIPT (n=50). It is a “top 100” substance (IFRA, pers. comm.2010).

METHYL DIHYDROJASMONATE	
CAS # 24851-98-7	
EC # 246-495-9	
Methyl 2-(3-oxo-2-pentyl cyclopentyl) acetate	
Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester; Kharismal; MDJ; Methyl (3-oxo-2-pentylcyclopentyl)acetate; Methyl 3-oxo-2-pentylcyclopentane ethanoate; Hedione	

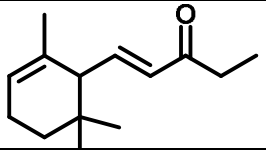
Current regulation: /

Clinical data:

In the Frosch 2002 b study, 3 of 1606 consecutive patients (0.2%) showed positive reactions to hedione (5% pet.) (17). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% hedione in pet., tested in 100 consecutive patients in Belfast, were observed (15).

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). An older RIFM review exists (128) citing 1 negative human maximisation test (n=25).

METHYL IONONE (mixture of isomers)	
CAS # 1335-46-2	
EC # 215-635-0	

1-(2,6,6-Trimethyl-1-cyclohex-2-enyl)pent-1-en-3-one	
6-Methylionone	

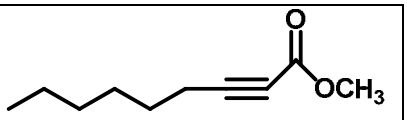
Current regulation: /

Clinical data:

See METHYLIONANTHEME for one clinical case report. Regarding methyl ionone gamma, the Frosch 1995 dose-finding pilot study found no positive reaction to 1% and 5% of this substance in pet., tested in 100 consecutive patients in Belfast (15).

Additional information:

It is a "top 100" substance (IFRA, pers. comm.2010). A RIFM review is available (171).

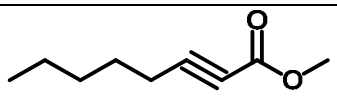
METHYL OCTINE CARBONATE	
CAS # 111-80-8	
EC #	
Methyl 2-octynoate	
Methyl 2-Nonynoate, MOC	

Current regulation: Annex III, part 1, n°173

Clinical data:

English and Rycroft reported a case of a 19-year-old laboratory technician working in the fragrance industry, who developed hand dermatitis after contact with methyl heptine and methyl octane carbonates; patch testing was strongly positive to both compounds at 1% in MEK (172).

Additional information: /

METHYL 2-OCTYNOATE	
CAS # 111-12-6	
EC # 203-836-6	
Methyl oct-2-ynoate	
M2O; Methyl heptin carbonate; Folione; Methyl hept-1-yne-1-carboxylate; Methyl pentylacetylenecarboxylate; NSC 72098; Vert de violette artificial	

Current regulation: Annex III, part 1, n° 89

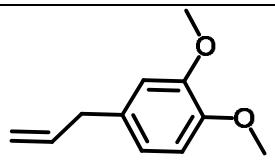
Clinical data:

In the "background information" section of the 1999 opinion, methyl 2-octynoate is classified as "less frequently reported allergen"; with only single cases of reported contact allergy, but the observation of this compound being a strong sensitizer according to IFRA (33), as also reported by Hostynek and Maibach (173)

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.3% (95% CI: 0.1 – 0.49%) positive reactions in 2401 consecutively PTed patients (1% pet.) (4). The IVDK 2010 study, n=1 weak positive reaction was observed in 988 patients tested with the compound (7). In the Groningen 2009 study, n=1, i.e. 0.3% (95% CI: 0.01 – 1.7%)

had positive reactions to this allergen, tested at only 2% pet. (6). In a previous case report of a fragrance laboratory assistant with work-related ACD both methyl heptin and methyl octin carbonate had been found sensitisers – probably due to their very similar chemical structure (172). In a recent bi-centric study with 350 eczema patients who were consecutively tested with 1% and 2% M2O in pet.; 0.8% positive reactions were observed. However, in 3 additional cases active sensitisation, with first reactions appearing 2 to 4 weeks after the patch test, and prompt reactions in the 2 cases repeat-patch tested, was observed (174).

Additional information: /

METHYL EUGENOL	
CAS # 93-15-2	
EC # 202-223-0	
1,2-Dimethoxy-4-(prop-2-enyl)benzene	
4-Allylveratrole; Eugenyl methyl ether extra; 1,2-Dimethoxy-4-allylbenzene; 1,3,4-Eugenol methyl ether; 1-(3,4-Dimethoxyphenyl)-2-propene; 1-Allyl-3,4-dimethoxybenzene; 3,4-Dimethoxy-1-(2-propenyl)benzene; 3,4-Dimethoxyallylbenzene; 3-(3,4-Dimethoxyphenyl)propene; 4-Allyl-1,2-dimethoxybenzene; Benzene, 4-allyl-1,2-dimethoxy-; Chavibetol methyl ether; Ent 21040; Eugenol methyl ether; Eugenyl methyl ether; Methyl eugenol ether; Methyl eugenyl ether; Methylchavibetol; NSC 209528; NSC 8900; O-Methyleugenol; Veratrole methyl ether; Veratrole, 4-allyl-	

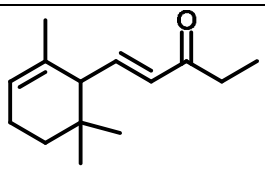
Current regulation: Annex II, 451

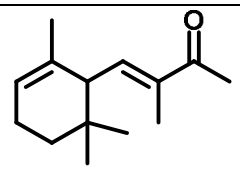
Clinical data:

In a previous study by Larsen et al (2002 c), 1.8% of patients with contact allergy to fragrance ingredients reacted positively to this compound (1).

Additional information:

Quote from the SCCS-opinion 0373/00: “Methyleugenol should not be intentionally added as a cosmetic ingredient. However, when fragrance compounds containing methyleugenol naturally present in essential oils are used as components in cosmetic products, the highest concentration of methyleugenol in the finished products must not exceed 0.01 % in fine fragrance, 0.004 % in eau de toilette, 0.002 % in a fragrance cream, 0.0002 % in other leave-on products and in oral hygiene products, and 0.001% in rinse-off products.” (The reason is genotoxicity and carcinogenicity).

METHYLIONANTHEME	
CAS # 55599-63-8	
EC #	
(1E)-2-Methyl-1-(2,6,6-trimethyl-2-cyclohexen-1-yl)-1-penten-3-one mixt. with **(3E)-3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one**	

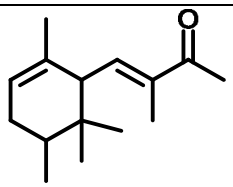
8-Methyl-α-ionone-10-methyl-α-ionone mixt.; Iralia Mixture	

Current regulation: …

Clinical data:
One case of ACD has been reported, caused by an E.d.C. (175).

Additional information:

Patented by GIVAUDAN SA 1933, is composed of isomeric n-methylionones and iso-methylionones. Methylionone has CAS # 1335-94-0 (not in CosIng) and 1335-46-2 (METHYL alpha-IONONE ISOMERS); other names: Methyl-alpha-cyclocitrilydenacetone; Iralia; Isoaldeine (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41456, last accessed 2010-07-14).

5-METHYL-alpha-IONONE	
CAS # 79-69-6	
EC # 201-219-6	
4-(2,5,6,6-Tetramethyl-2-cyclohexen-1-yl)-3-buten-2-one	
Methyl-α-Ionone; 6-Methyl-α-ionone; α-Irone	

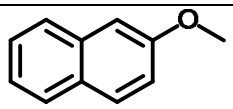
Current regulation: /

Clinical data:

In the Frosch 2002 b study, 5 of 1606 consecutive patients (0.3%) showed positive reactions to alpha-irone (10% pet.) (17).

Additional information:

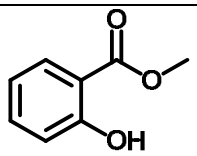
A RIFM review is available (176), citing a (negative) human maximisation test and the study results quoted.

METHYL beta-NAPHTHYL ETHER	
CAS # 93-04-9	
EC # 202-213-6	
2-Methoxynaphthalene	
beta-Naphthyl methyl ether; methyl 2-naphthyl ether; Nerolin (old); NSC 4171; Yara yara; β-Methoxynaphthalene; β-Naphthol methyl ether; β-Naphthyl methyl ether; 2-Methoxynaphthalene; Methyl β-naphthyl ether; 2-Naphthol methyl ether; 2-Naphthyl methyl ether; 6-Methoxy-2-naphthalene	

Current regulation: /

Clinical data: /

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

METHYL SALICYLATE	
CAS # 119-36-8	
EC # 204-317-7	
Methyl 2-hydroxybenzoate	
Other names: Salicylic acid, methyl ester; 2-(Methoxycarbonyl)phenol; 2-Carbomethoxyphenol; 2-Hydroxybenzoic acid methyl ester; Analgit; Anthrapole ND; Ben Gay; Exagien; Flucarmit; Methyl ester of 2-hydroxy benzoic acid; Methyl o-hydroxybenzoate; Methyl salicylate; NSC 8204; Wintergreen oil; o-Hydroxybenzoic acid methyl ester; “Oil of wintergreen”	

Current regulation: /

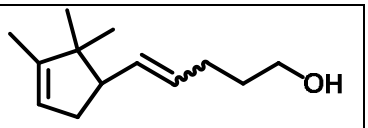
Clinical data:

The deGroot 2000 study yielded 7 positive reactions to methyl salicylate (2% pet.) in 1825 consecutive patients (12).

A case of ACD following the application of a compress bandage containing methyl salicylate has been reported, using 2% “o.o.” as PT concentration; the dose per area of methyl salicylate in the occlusive bandage was not reported (177). A similar case was reported in 1977, positive to 2% methyl salicylate in olive oil, with elicitation of pruritus and erythema after oral ingestion of acetyl salicylic acid (178).

Additional information:

A RIFM review is available (179) providing an overview on 3 human sensitisation experiments (e.g., the HRIPT) which were all negative, and clinical data. In a number of older PT studies, positive test results were seen in 6 of 4600, 3 of 183, 3 of 241, 17 of 585, 1 of 70, all employing a test concentration of 2%, usually in pet., according to above review. Methyl salicylate may occur in topical analgesic (OTC) medications, in Germany, for instance, in ”Camphopin® Salbe“ („Rote Liste 2010“).

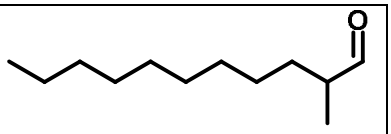
3-METHYL-5-(2,2,3-TRIMETHYL-3-CYCLOPENTENYL)PENT-4-EN-2-OL	
CAS # 67801-20-1	
EC # 267-140-4	
3-Methyl-5-(2,2,3-trimethyl-1-cyclopent-3-enyl)pent-4-en-2-ol	
3-Methyl-5-(2,2,3-trimethyl-3-cyclopenten-1-yl)-4-penten-2-ol; 3-Methyl-5-(2,2,3-trimethylcyclopent-3-enyl)pent-4-en-2-ol; Ebanol	

Current regulation: /

Clinical data:

In the Larsen 2001 study, 1 of 178 patients with known contact allergy to fragrance ingredients exhibited a positive PT reaction to "MTCP", tested 5% pet. (19). In the An 2005 study, 12 of 422 consecutive patients, i.e., 2.8%, had positive reactions to “ebanol”, tested at 5% (13).

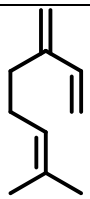
Additional information: /

METHYLUNDECANAL	
CAS # 110-41-8	
EC # 203-765-0	
2-Methylundecanal	
Aldehyde c-12 mna; undecenal, 2-methyl-; 2-Methyl-1-undecanal; Aldehyde M.N.A.; Methyl n-nonyl acetaldehyde; Methylnonylacetaldehyde; NSC 46127	

Current regulation: /

Clinical data: /

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

MYRCENE	
CAS # 123-35-3	
EC # 204-622-5	
7-Methyl-3-methylideneocta-1,6-diene	
2-Methyl-6-methylene-2,7-octadiene; 7-Methyl-3-methylene-1,6-octadiene; NSC 406264; β-Geraniolene; β-Myrcene	

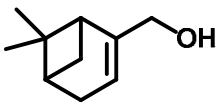
Current regulation: /

Clinical data:

In a clinical study in 6 European centres, including 1511 consecutive patients, 1 patient had a positive reaction to oxidized myrcene (65).

Additional information:

Myrcene autoxidizes spontaneously and rapidly at air exposure. In experimental studies on beta-myrcene an EC3 value of 4.3% was seen for a sample air-exposed 10 weeks (Sköld M. Contact allergy to autoxidized fragrance terpenes (180).

MYRTENOL	
CAS # 515-00-4	
EC # 208-193-5	

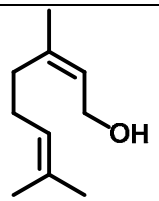
(7,7-Dimethyl-4-bicyclo[3.1.1]hept-3-enyl)methanol
(-)-Pin-2-ene-10-ol; 2-Pinen-10-ol; (6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)methanol; (±)-Myrtenol; 6,6-Dimethyl-2-(hydroxymethyl)bicyclo[3.1.1]hept-2-ene; NSC 408846; α-Pinene-10-ol

Current regulation: /

Clinical data: /

Additional information:

A RIFM review exists (181), citing 2 of 3 HRIPT studies with 1 case of sensitisation to myrtenol each.

NEROL	
CAS # 106-25-2	
EC # 203-378-7	
(2Z)-3,7-Dimethylocta-2,6-dien-1-ol	
2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-; (Z)-3,7-Dimethyl-2,6-octadien-1-ol; (Z)-Geraniol; (Z)-Nerol; 2-cis-3,7-Dimethyl-2,6-octadien-1-ol; 3,7-Dimethyl-cis-2,6-octadien-1-ol; Nerol 900; Neryl alcohol; cis-3,7-Dimethyl-2,6-octadien-1-ol; cis-Geraniol; β-Nerol; cis-geraniol – i.e., isomeric to geraniol	

Current regulation: /

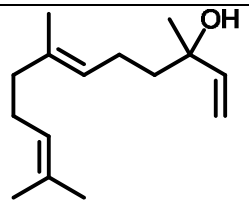
Clinical data:

In the Larsen 2002 c study, 6.0% of the fragrance sensitive patients reacted positively to 5% in pet. (1).

Additional information:

A RIFM review is available (182) citing (negative) human sensitisation experiments, an older study from Japan and the Larsen 2002 c study (see above).

Regarding autoxidation studies – see geraniol.

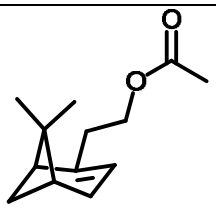
Nerolidol (isomer not specified)	
CAS # 7212-44-4	
EC # 230-597-5	
3,7,11-Trimethyl-1,6,10-odecatrien-3-ol	
Nerolidol; (±)-Nerolidol; FCI 119b; Nerodilol	

Current regulation: /

Clinical data: /

Additional information:

RIFM review is available (183) citing the occurrence of "3 positive reactions in 2273 patients". Another RIFM review is available on cis-nerolidol (184), mentioning that no data on this compound are available.

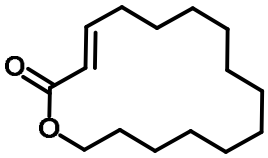
NOPYL ACETATE	
CAS # 128-51-8	
EC # 204-891-9	
2-(7,7-Dimethyl-4-bicyclo[3.1.1]hept-3-enyl)ethyl acetate	
2-Norpinene-2-ethanol, 6,6-Dimethyl-, acetate; Bicyclo[3.1.1]hept-2-ene-2-ethanol, 6,6-dimethyl-, acetate; 2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl acetate; 7,7-Dimethylbicyclo[3.1.1]hept-2-ene-2-ethanol acetate; Citroviol; NSC 1286; NSC 404963; Nopol acetate; Nopyl acetate	

Current regulation: /

Clinical data:

The DeGroot 1985 study identified 2 (1.1%) positive reactions among 179 patients using a 25% PT preparation of this compound – reactions may have at least partly been due to an "excited back syndrome" and thus a limited evidence (25).

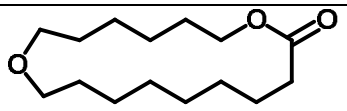
Additional information: /

OXACYCLOHEXADECENONE	
CAS # 34902-57-3	
EC # 609-040-9	
(3E)-Oxacyclohexadec-3-en-2-one	
Globalide; Oxacyclohexadecen-2-one	

Current regulation: /

Clinical data: /

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

OXALIDE	
CAS # 1725-01-5	
EC # 217-033-3	
1,8-Dioxacycloheptadecan-9-one	
Nonanoic acid, 9-[(6-hydroxyhexyl)oxy]-, o-lactone; 10-Oxa-16-hexadecanolide; Oxalide; Oxalide T	

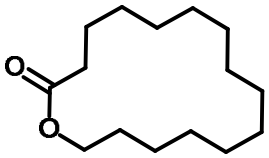
Current regulation: /

Clinical data:

In the Larsen 2001 study, none of 178 patients with known contact allergy to fragrance ingredients exhibited a positive PT reaction to "10-oxahexadecanolide", tested 5% pet. (19).

Additional information:

A RIFM review is available (128), citing a negative maximisation test (n=29).

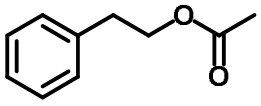
PENTADECALACTONE	
CAS # 106-02-5	
EC # 203-354-6	
1-Oxacyclohexadecan-2-one	
Pentadecanoic acid, 15-hydroxy-, ξ-lactone; 1,15-Pentadecanolide; 15-Hydroxypentadecanoic acid lactone; 15-Pentadecanolide; 15-Pentadodecanolactone; 2-Pentadecalone; CPE 215; Cyclopentadecanolide; Exaltolide; Macrolide Supra; Muskalactone; NSC 36763; Pentadecalactone; Pentadecanolactone; Pentadecanolide; Pentalide; Thibetolide; cpd Supra; ω-Pentadecalactone; angelica lactone; hexaltolide	

Current regulation: /

Clinical data: /

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). The substance has been used for clinical olfactory testing in the 60ies under the name of exaltolide.

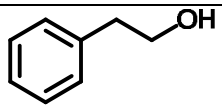
PHENETHYL ACETATE	
CAS # 103-45-7	
EC # 203-113-5	
2-Phenylethyl acetate	
Acetic acid, phenethyl ester ; Phenethyl alcohol, acetate; 2-Phenethyl acetate; 2-Phenylethyl acetate; Benzylcarbinyl acetate; NSC 71927; Phenethyl acetate; Phenylethyl ethanoate; β-Phenethyl acetate; β-Phenylethanol acetate; β-Phenylethyl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

Current regulation: /

Clinical data: /

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). Exposure via plants (*Tanacetum parthenium*) is possible (185).

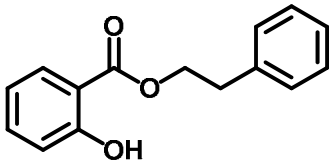
PHENETHYL ALCOHOL	
CAS # 60-12-8	
EC # 200-456-2	
2-Phenylethanol	
Phenethyl alcohol; (2-Hydroxyethyl)benzene; 2-Phenethanol; 2-Phenethyl alcohol; 2-Phenyl-1-ethanol; 2-Phenylethyl alcohol; Benzyl carbinol; Ethanol, 2-phenyl-; NSC 406252; PEA; Phenethanol; Phenethylol; Phenylethanol; Phenylethyl alcohol;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

Current regulation: /

Clinical data:

The DeGroot 1985 study identified 1 (0.6%) positive reactions among 179 patients using a 25% PT preparation of phenylethyl alcohol (25). In the Frosch 1995 dose-finding pilot study, no positive reaction to this compound, tested 1% pet. in 100 consecutive patients in Odense, DK, was observed (15).

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

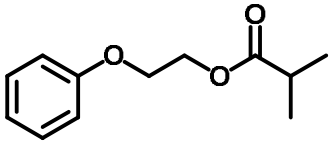
PHENETHYL SALICYLATE	
CAS # 87-22-9	
EC # 201-732-5	
2-Phenylethyl 2-hydroxybenzoate	
Salicylic acid, phenethyl ester; 2-Phenylethyl salicylate; Benzylcarbinyl salicylate; NSC 72035; Phenethyl salicylate	

Current regulation: /

Clinical data: /

Additional information:

A RIFM review exists (186), quoting a negative human maximisation test and a number of animal experiments, including cross-sensitisation experiments with benzyl salicylate. One LLNA study is reported yielding an EC3 value of 2.1%.

PHENOXYETHYL ISOBUTYRATE	
CAS # 103-60-6	
EC # 203-127-1	
2-Phenoxyethyl 2-methylpropanoate	
Isobutyric acid, 2-phenoxyethyl ester; Ethanol, 2-phenoxy-, isobutyrate; 2-Phenoxyethyl isobutyrate; NSC 227210; NSC	

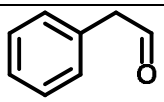
406209; Phenoxyethyl isobutyrate; β-Phenoxyethyl isobutyrate	

Current regulation: /

Clinical data: /

Additional information:

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

PHENYLACETALDEHYDE	
CAS # 122-78-1	
EC # 204-574-5	
2-Phenylacetaldehyde	
Benzylcarboxaldehyde; Hyacinthin; NSC 406309; Phenacetaldehyde; Phenylacetaldehyde; Phenylacetic aldehyde; Phenylethanal; α-Phenylacetaldehyde; α-Tolualdehyde; α-Toluic aldehyde	

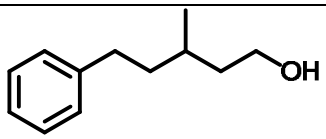
Current regulation: /

Clinical data:

In the Malten 1984 study, 1.1% of 182 patients displayed a positive PT reaction to phenylacetaldehyde 2% pet. (24). In a case report, Sanchez-Politta et al. describe a 26-year-old worker in a perfume factory, who suffered from a spill of pure phenylacetaldehyde and became sensitised, as proven by positive patch tests with 0.5%, 1% and 2% (10 healthy controls negative) (187).

Additional information:

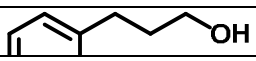
SCCS opinion: 1153/08 - Opinion on "Dermal Sensitization Quantitative Risk Assesment " (QRA: Citral, farnesol and phenylacetaldehyde)

PHENYLISOHEXANOL	
CAS # 55066-48-3	
EC # 259-461-3	
3-Methyl-5-phenylpentan-1-ol	
3-Methyl-5-phenyl-1-pentanol; 3-Methyl-5-phenylpentanol; 5-Phenyl-3-methylpentanol; Mefrosol; Phenoxanol	

Current regulation: /

Clinical data: /

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

PHENYLPROPANOL	

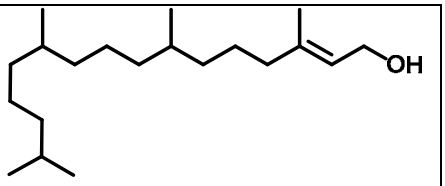
CAS # 122-97-4	
EC # 204-587-6	
3-Phenylpropan-1-ol	
(3-Hydroxypropyl)benzene; 1-Hydroxy-3-phenylpropane; 3-Benzenepropanol; 3-Hydroxy-1-phenylpropane; 3-Phenyl-1-propanol; 3-Phenyl-n-propanol; 3-Phenylpropanol; 3-Phenylpropyl alcohol; Dihydrocinnamyl alcohol; Hydrocinnamic alcohol; Hydrocinnamyl alcohol; NSC 16942; γ-Phenylpropanol; γ-Phenylpropyl alcohol; Phenethyl Carbinol	

Current regulation: /

Clinical data:

The Larsen 2002 c study yielded 0.9% positive reactions in 218 patients with contact allergy to fragrance ingredients (1).

Additional information: …

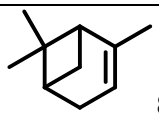
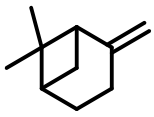
PHYTOL	
CAS # 150-86-7	
EC # 205-776-6	
(E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol	
Phytol; (7R,11R,2E)-Phytol; (E)-Phytol; (E,R,R)-Phytol; 3,7,11,15-Tetramethylhexadec-2-en-1-ol; trans-Phytol	

Current regulation: /

Clinical data:
/

Additional information:

Phytol is a main constituent of Jasmin abs. with 7.4% reported content (17). In a human maximization study involving 25 subjects, there was one case of contact sensitization to 10% phytol (6900 µg/cm²), applied in petrolatum, as reported in a RIFM review (188).

alpha-PINENE and beta-PINENE	 80-56-8  127-91-3
CAS # 80-56-8 (alpha-Pinene); CAS # 127-91-3 (beta-Pinene)	
EC # 201-291-9 (alpha-Pinene; according to CAS service: 219-445-9); EC # 204-872-5 (beta-Pinene; according to CAS service: 245-424-9)	
2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene (80-56-8) **6,6-Dimethyl- 2-methylenebicyclo[3.1.1]heptane** (127-91-3)	

80-56-8: 2-Pinene; (±)-2-Pinene; (±)-α-Pinene; Acintene A; NSC 7727; PC 500; PC 500 (terpene); Sylvapine A; α-Pinene **127-91-3**: 2(10)-Pinene ; (±)-2(10)-Pinene; (±)-6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane; (±)-β-Pinene; 6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane; NSC 21447; NSC 406265; NSC 59190; Nopinen; Nopinene; PC 600; PC 600 (pesticide); Pseudopinen; Pseudopinene; Terebenthene; β-Pinene	

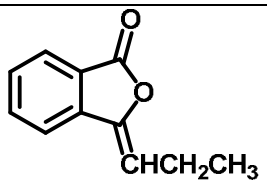
Current regulation: Annex III, part 1, n° 130 (Peroxide value less than 10 mmoles/L in substance)

Clinical data:

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

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

Additional information: /

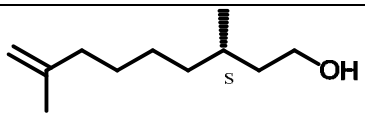
PROPYLIDENE PHTHALIDE	
CAS # 17369-59-4	
EC # 241-402-8	
3-Propylidene-2-benzofuran-1-one	
3-Propylidene-1(3H)-isobenzofuranone; 3-Propylidenephthalide; Celeriax; Propylidenephthalide	

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

Clinical data:

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

Additional information: /

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

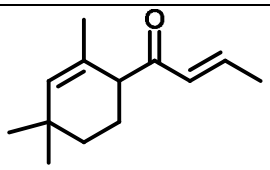
α-Citronellol	

Current regulation: /

Clinical data: / (see below)

Additional information:

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

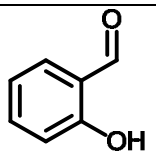
trans-ROSE KETONE-5	
CAS # 39872-57-6	
EC # 254-663-8	
(2E)-1-(2,4,4-Trimethylcyclohex-2-en-1-yl)but-2-en-1-one	
alpha-Isodamascone; trans-2,4,4-Trimethyl-1-crotonyl-2-cyclohexene; (E)-1-(2,4,4-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one	

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

Clinical data: /

Additional information:

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

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

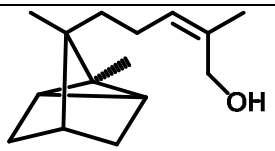
Current regulation: /

Clinical data:

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

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

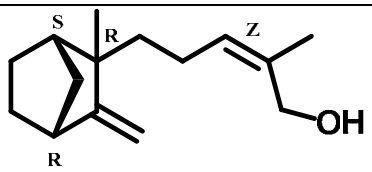
Additional information: Along with other derivates of salicylic acid, salicylaldehyde is found in the bark of several trees, such as willow or aspen, and can cause allergic contact dermatitis by this exposure (196).

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

Current regulation: /

Clinical data: / (see beta-santalol)

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

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

Current regulation: /

Clinical data:

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

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

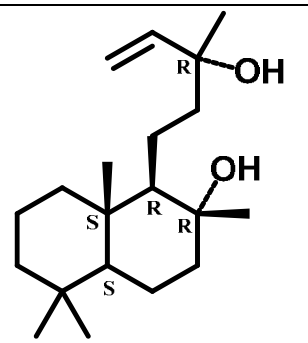
Additional information: "There is no one CAS number for the mixture. The alpha form has a CAS No. 115-71-9 and the beta form is 37172-32-0 (this # is trans-.beta.-santalol). There was no reported use of these materials in the last two IFRA Surveys (8 years total)" (A.M. Api, pers. comm., 2010).

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

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

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

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

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

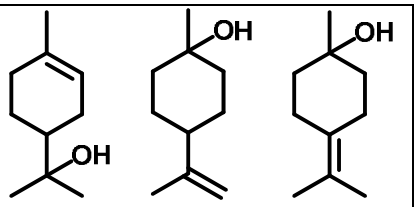
Current regulation: /

Clinical data: /

Additional information:

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

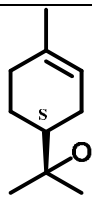
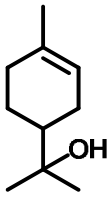
0986/06 - Opinion on Sclareol (sensitisation only) (http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_056.pdf)

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

Current regulation: /

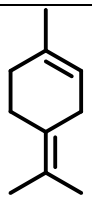
Clinical data:
A RIFM review is available (200), citing negative human induction studies and one clinical study “Takenaka 1986”, finding 4 of 312 patients with 0.05% to 0.5% terpineol in a cream base and in ethanol, resp., and 2 negative clinical studies of limited size. In the Frosch 1995 dose-finding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

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

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

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

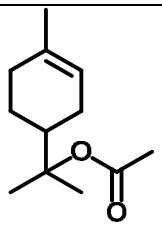
Additional information: see also terpineol (mixture of isomers). Comments on turpentine under pinene.

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

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

gave a positive result (negative in 10 controls) (203). Eleven patients sensitised to tea tree oil showed positive reactions to alpha-terpinene, terpinolene and ascaridol (204).

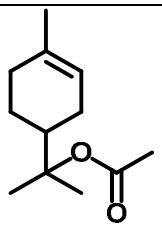
Additional information: It is a “top 100” substance (IFRA, pers. comm. 2010)

TERPINEOL ACETATE (Isomer mixture)	
CAS # 8007-35-0	
EC # 232-357-5	
4-Methyl-1-propan-2-yl-1-cyclohex-2-enyl acetate	
Terpinyl acetate	

Current regulation: /

Clinical data:
In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% terpinyl acetate in pet., tested in 106 consecutive patients in Barcelona, were observed (15)

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

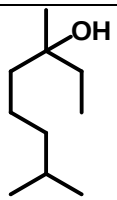
alpha-TERPINYL ACETATE	
CAS # 80-26-2	
EC # 201-265-7	
2-(4-Methyl-1-cyclohex-3-enyl)propan-2-yl acetate	
3-Cyclohexene-1-methanol, α,α,4-trimethyl-, acetate; p-Menth-1-en-8-ol, acetate; (±)-α-Terpineol acetate; (±)-α-Terpinyl acetate; 2-(4-Methyl-3-cyclohexen-1-yl)-2-propyl acetate; Terpinyl acetate;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

Current regulation: /

Clinical data:

The DeGroot 1985 study identified no positive reactions among 179 patients using a 10% PT preparation of “terpinyl acetate” (25).

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

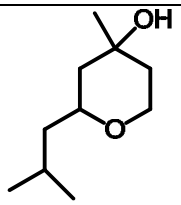
Tetrahydrolinalool	
CAS # 78-69-3	
EC # 201-133-9	
3,7-Dimethyloctan-3-ol	
2,6-Dimethyl-6-octanol; 3,7-Dimethyloctan-3-ol; Linalool tetrahydride; NSC 128151; Tetrahydrolinalool	

Current regulation: /

Clinical data:
/

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

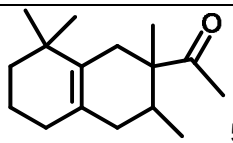
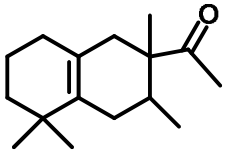
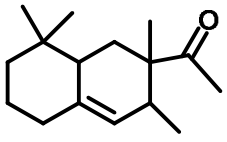
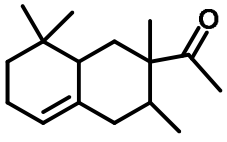
review is available (205) quoting 1 negative human maximisation test.

TETRAHYDRO-METHYL-METHYLPROPYL)-PYRAN-4-OL	
CAS # 63500-71-0	
EC # 405-040-6	
4- Methyl-2-(2-methylpropyl)tetrahydro-2H-4-pyranol	
2-(2-Methylpropyl)-4-hydroxy-4-methyltetrahydropyran; 2-Isobutyl-4-hydroxy-4-methyltetrahydropyran; 2-Isobutyl-4-methyltetrahydropyran-4-ol; 4-Hydroxy-4-methyl-2-(2-methylpropyl)tetrahydropyran; Florosa; Rozanol	

Current regulation: /

Clinical data: /

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

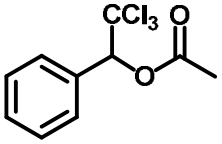
TETRAMETHYL ACETYLOCTAHYDRONAPHTHALENES	 54464-57-2
CAS # 54464-57-2 / 54464-59-4 / 68155-66-8 / 68155-67-9	
EC # 259-174-3 / 259-175-9 / 268-978-3 / 268-979-9	
1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)-ethanone (54464-57-2) **1-(1,2,3,4,5,6,7,8-Octahydro-2,3,5,5-tetramethyl-2-naphthalenyl)-ethanone** (54464-59-4) **1-(1,2,3,5,6,7,8,8a-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)-ethanone** (68155-66-8) **1-(1,2,3,4,6,7,8,8a-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)-ethanone** (68155-67-9)	 54464-59-4  68155-66-8  68155-67-9
54464-57-2: 1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)ethanone; 1',2',3',4',5',6',7',8'-Octahydro-2',3',8',8'-tetramethyl-2'-acetonaphthone; 7-Acetyl-1,2,3,4,5,6,7,8-octahydro-1,1,6,7-tetramethylnaphthalene; Amberonne; Ambralux; Iso Ambois Super; Iso-E Super; Isocyclemone E; OTNE; Orbitone	

Current regulation: /

Clinical data:
In the Frosch 2002 a study, 0.2% of 1855 consecutive patients reacted to the compound (brand name mentioned: „Iso E. Super“, 5% pet.) (16). In the Frosch 1995 dose-finding pilot study, 1 positive reaction both to 1% and 5% “Iso E Super ®” in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15). The Larsen 2001 study yielded 1.7% positive reactions (5% pet.) in 178 patients with known contact allergy to fragrance ingredients (19).

Additional information: According to CosIng: "Mixture of isomers: 1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8-tetramethyl-2-naphthyl)ethan-1-one; 1-(1,2,3,4,5,6,7,8-Octahydro-2,3,5,5-tetramethyl-2-naphthyl)ethan-1-one; 1-(1,2,3,5,6,7,8,8a-Octahydro-2,3,8,8-tetramethyl-2-naphthyl)ethan-1-one (68155-67-9); 1-(1,2,3,4,6,7,8,8a-Octahydro-2,3,8,8-tetramethyl-2-naphthyl)ethan-1-one (68155-66-8) " (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40504, last accessed 2009-11-11).

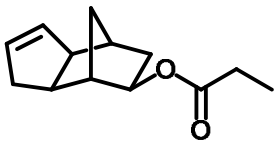
It is a "top 100" substance (IFRA, pers. comm. 2010)

TRICHLOROMETHYL PHENYL CARBINYL ACETATE	
CAS # 90-17-5	
EC # 201-972-0	
2,2,2-Trichloro-1-phenylethyl acetate	
Benzenemethanol, α-(trichloromethyl)-, acetate; Benzyl alcohol, α-(trichloromethyl)-, acetate (Trichloromethyl)phenylcarbinyl acetate; (±)-α-(Trichloromethyl)benzyl acetate; 2-Acetoxy-1,1,1-trichloro-2-phenylethane; Crystal rose; NSC 165582; Rosacetol; Rosephenone; Rosetone; Rosone; α-(Trichloromethyl)benzyl acetate	

Current regulation: /

Clinical data: /

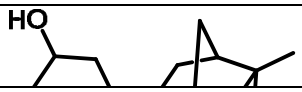
Additional information: It is a "top 100" substance (IFRA, pers. comm. 2010)

TRICYCLODECENYL PROPIONATE	
CAS # 17511-60-3	
EC # 241-514-7	
3α,4,5,6,7,7α-Hexahydro-4,7-methano-1H-inden-6-yl propionate	
4,7-Methano-1H-inden-6-ol, 3a,4,5,6,7,7a-Hexahydro-, propanoate; 4,7-Methanoinden-6-ol, 3a,4,5,6,7,7a-Hexahydro-, propionate; Cyclaprop; Florocyclene; Greenyl propionate; Tricyclo(5.2.1.02,6)dec-3-en-8-yl propionate.	

Current regulation: /

Clinical data: /

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

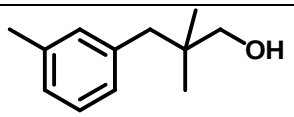
3-(5,5,6-TRIMETHYLBICYCLO[2.2.1]HEPT-2-YL)-CYCLOHEXAN-1-OL	

CAS # 3407-42-9	
EC # 222-294-1	
3-(5,5,6-Trimethyl-6-bicyclo[2.2.1]heptanyl)cyclohexan-1-ol	
3-(5,5,6-Trimethyl-2-norbornyl)-cyclohexanol; 3-(5,5,6-Trimethylbicyclo[2.2.1]hept-2-yl)cyclohexan-1-ol; 3-Hydroxy-1-(5-isocamphyl)cyclohexane; Sandela	

Current regulation: /

Clinical data: /

Additional information: part of “synthetic sandalwood oil”.

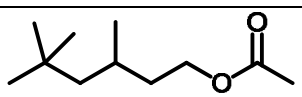
TRIMETHYL-BENZENEPROPANOL (Majantol)	
CAS # 103694-68-4	
EC # 403-140-4	
2,2-Dimethyl-3-(3-methylphenyl)propan-1-ol	
2,2-Dimethyl-3-(3-tolyl)propan-1-ol; 3-(2,2-Dimethyl-3-hydroxypropyl)toluene	

Current regulation: /

Clinical data:

In the Larsen 2002 c study, majantol (conc. not given, elsewhere reported as 5% pet.) caused positive PT reactions in 3.2% of patients with known contact allergy to fragrance ingredients. In a later study by the IVDK, 0.5% (95% CI: 0.3 – 0.7%) consecutive patients displayed a positive reaction to majantol 5% pet. (206). In the IVDK 2010 study, majantol was tested both in n=2189 consecutive patients, yielding 0.36 % (95% CI: 0.12—0.60%) positive reactions, and in the context in a special series, applied in an aimed fashion to n=4972 patients, yielding 0.76% (95% CI: 0.49—1.03%) (standardised) positive reactions (7). In a recent study from Copenhagen, DK, 6 of 722 patients tested with this compound were found positive, 2 of these to material used earlier provided by Symrise, 4 to material by Allmiral/Hermal/Trolab used later instead. There was no significant difference between these proportions obtained with batches of majantol from different production processes (207).

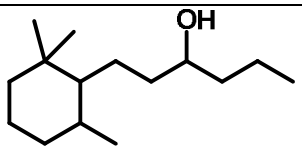
Additional information: /

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

Current regulation: /

Clinical data: /

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

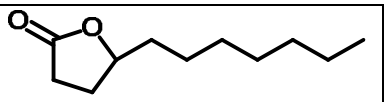
TRIMETHYL-PROPYLCYCLOHEXANEPROPANOL (TMCH)	
CAS # 70788-30-6	
EC # 274-892-7	
1-(2,2,6-Trimethylcyclohexyl)hexan-3-ol	
Other names: 2,2,6-Trimethyl-alpha-propylcyclohexanepropanol (REACH, EINECS); .alpha.-Propyl-2,2,6-trimethyl-cyclohexanepropanol; 6-(2,2,6-Trimethylcyclohexyl)-4-hexanol; Finotimber;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

Current regulation: /

Clinical data:

In the Larsen 2001 study, none of 178 patients with contact allergy to fragrance ingredients reacted positively to this ingredient, PTed at 5% pet. (19).

Additional information: /

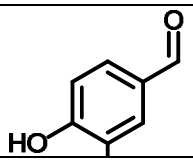
gamma-UNDECALACTONE	
CAS # 104-67-6	
EC # 203-225-4	
5-Heptyltetrahydrofuran-2-one	
Undecanoic acid, 4-hydroxy-, γ-lactone; (RS)-γ-Undecalactone; (±)-γ-Undecalactone; 4-Hydroxyundecanoic acid lactone; 4-Undecanolide; 5-Heptyldihydro-2(3H)-furanone; NSC 406421; NSC 46118; NSC 76413; Neutralizing agent 350120-1; Peach lactone; Peche Pure; Persicol; γ-(n-Heptyl)-γ-butyrolactone; γ-Heptyl-γ-butyrolactone; γ-Heptylbutyrolactone; γ-Undecalactone; γ-Undecanolactone; γ-Undecanolide; γ-n-Heptylbutyrolactone	

Current regulation: /

Clinical data: /

Additional information:

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

VANILLIN	
CAS # 121-33-5	
EC # 204-465-2	

4-Hydroxy-3-methoxybenzaldehyde	
2-Methoxy-4-formylphenol; 3-Methoxy-4-hydroxybenzaldehyde; 4-Formyl-2-methoxyphenol; 4-Hydroxy-5-methoxybenzaldehyde; 4-Hydroxy-m-anisaldehyde; H 0264; Lioxin; NSC 15351; NSC 403658; NSC 48383; Rhovanil; Vanillaldehyde; Vanillic aldehyde; Vanillum; m-Methoxy-p-hydroxybenzaldehyde; p-Hydroxy-m-methoxybenzaldehyde; p-Vanillin	

Current regulation: /

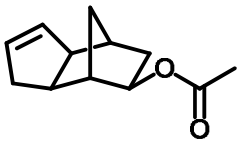
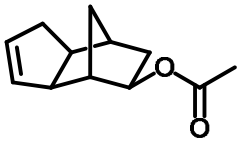
Clinical data:

In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 1 positive reaction to vanillin was observed (3). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=1 (0.1%) positive reaction to vanillin 10 % pet. (22). The IVDK 2010 study, n=10, i.e., 0.19% (95% CI: 0.07 – 0.32%; percentages standardised for age and sex) of 4377 patients PTed reacted to the compound, tested 10% pet. (7). In n=102 patients with a positive reaction to MPR, 19 compounds of this natural mixture were tested, among these, vanillin, to which none reacted positively (208). In 21 patients with contact allergy to propolis, 2 also reacted to vanillin (10% pet.) (209).

A 13-year-old girl with recurrent (peri-)cheilitis after application of a vanilla lip salve tested strongly positive to this salve (as is), “Vanilla 10% pet.” (unclear, whether natural extract or vanillin) and MPR (210). Trattner/David identified 1 / 641 consecutive patients with positive reaction to vanillin (31).

Additional information:

Naturally occurring in the fruit of *Vanilla planifolia* after a fermentation process, in styrax, clove oil, potatoes, wood, including Myroxylon pereirae resin, and other material (53). Nowadays, vanillin is synthesised from eugenol, guajakol and lignin residues from paper production, however, not fully achieving the subtle scent and taste of the natural material (53). It is a “top 100” substance and classified as R43 (IFRA, pers. comm. 2010).

VERDYL ACETATE	
CAS # 2500-83-6/ 5413-60-5	
EC # 219-700-4 / 226-501-6	
3a,4,5,6,7,7a-Hexahydro-4,7-methanoinden-6-yl acetat (2500-83-6)	2500-83-6
3a,4,5,6,7,7a-Hexahydro-4,7-methano-1H-inden-5-yl acetat (5413-60-5)	
2500-83-6: 4,7-Methano-1H-inden-5-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; 4,7-Methanoinden-5-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; NSC 142428; NSC 94573	5413-60-5
5413-60-5: 4,7-Methano-1H-inden-6-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; 4,7-Methanoinden-6-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; 4,7-Methano-3a,4,5,6,7,7a-hexahydroinden-6-yl acetate; 8-Acetoxytricyclo[5.2.1.02,6]dec-3-ene; Greenyl acetate;	

Herbaflorat; Jasmacyclene; NSC 6598	

Current regulation: /

Clinical data: /

Additional information:

In CosIng, both above CAS numbers are listed under “verdyl acetate” (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41289, last accessed 2010-07-19).

In the CAS, there are 2 separate entries; moreover, there are 2 separate RIFM reviews:

- # 2500-83-6: Other names: Tricyclo[5.2.1.02,6]dec-4-en-8-yl acetate (REACH, EINECS, INCI Name according to CAS); 3a,4,5,6,7,7a-Hexahydro-4,7-methanoinden-6-yl Acetate; Tricyclodecen-4-yl 8-Acetate. It is a “top 100” substance (IFRA, pers. comm. 2010). A RIFM review is available, stating that “no data is available” regarding the skin sensitising properties of the substance (211).
- # 5413-60-5: Other names: 3a,4,5,6,7,7a-hexahydro-4,7-methanoinden-6-yl acetate (REACH, EINECS, INCI Name according to CAS), 4,7-Methano-3a,4,5,6,7,7a-hexahydroinden-6-yl acetate; 4,7-Methanoinden-6-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; 8-Acetoxytricyclo[5.2.1.02,6]dec-3-ene; Tricyclodecenyl acetate; Greenyl acetate; Herbaflorat; Jasmacyclene; NSC 6598; Verdyl acetate. It is a “top 100” substance (IFRA, pers. comm. 2010). A RIFM review is available (212), citing 2 negative human maximisation tests and 1 negative HRIPT.

Natural extracts / essential oils

Natural raw materials in terms of extracts are used in the fragrance and flavour industry for various reasons. Most importantly, several naturally occurring mixtures have a very complex composition and sensory nature which cannot (fully) be achieved by synthetic material. Moreover, several compounds cannot be synthesised at a competitive price, and the demand for perfumes based on natural materials is considerable (34).

The three main methods used to concentrate plant fragrance substances as essential oils comprise steam distillation, mechanical processes from the epicarp of Citrus fruits ("pressing") and dry distillation.A Essential oil is „obtained by steam distillation with addition of water in the still (hydrodistillation) or without addition of water in the still (directly by steam")(213). Essenctial oil of fruit juice is „obtained by from a fruit juice during its concntration or during UHT (flash pasteurization) treatment" (213). Cold pressed essential oil is „obtained by mechanical processes from the epicarp of the fruit of a Citrus, at ambient temperature"(213). Citrus peel oils, apart from distilled Citrus oils, are produced with varous methods (214). The oil consists of a high volume of volatile terpenes, mostly monoterpenes but also contains small amounts of non-volatile compounds such as dyes, waxes and furocoumarines.

The method of solvent extraction is generally applied in the separation of heat-labile materials or if an essential oil can only be obtained in very low yield, e.g. from blossoms. It is also used if the non-volatile components are desired for their fixative properties, e.g. in the preparation of resinoids from exudates. The most important extracts are termed: (i) concrete: an extract „obtained from a fresh plant natural raw material by extraction with a solvant"[18], containing not only volatile, but also a large proportion of non- volatile substances such as waxes; and (ii) absolute: „product, obtained by extraction with ethanol from a concrete, a floral pomade, a resinoid or a supercritical fluid extract. The ethanolic solution is generally cooled down and filtered in order to eliminate the «waxes»; the ethanol is then eliminated by distillation"[19]. Resinoids, used for their fixative properties, are „obtained from a dry plant natural raw material by extraction with a sovant"[20]. The products are usually highly viscous and thus might sometimes be diluted, e.g. with phthalates or benzyl benzoate. Oleoresins are extracts „of spice or aromatic herb" by „treating a natural raw material with a solvent, then, after filtration if necessary, the solvent is elimitated"[21].

Regarding clinical data in terms of contact allergy to fragrance ingredients, the main focus of case report or clinical studies regarding essential oils and natural extracts, respectively, is on general dermatological patients with complaints related to use of cosmetics etc. However, series of cases with occupational exposure to essential oils with occupational allergic contact dermatitis have also been reported (e.g., masseurs,

[18] ISO/DIS 9235

[19] ISO/DIS 9235

[20] ISO/DIS 9235

[21] ISO/DIS 9235

physiotherapists (215, 216), aromatherapists (217-221), beauticians doing massages (222); for further details, e.g., PT results with various essential oils, see original case reports. “Current Regulation” refers to the EU Cometics Directive only.

Catalogue of natural extracts / essential oils evaluated

ACORUS CALAMUS *ROOT OIL*	*CAS 84775-39-3; EC* 283-869-0
Calamus Oil; "Sweet Flag Oil"	*(Acorus calamus, ext. = INCI name)*

Current regulation: /

Clinical data:

The Rudzki 1976 study found no positive reaction in 200 patients to “calamus” essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=7 (8.1%) positive reactions to “calamus” essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Acorus calamus* L. (sweet flag calamus). Acorus Calamus Root Oil is an essential oil obtained from the rhizomes of the calamus, Acorus calamus L., Araceae. It contains beta-asarone (up to 96%, depending on ploidy, and with this, origin (34)), calamene (about 4%), calamol (about 3%) alpha-asarone (about 1%), camphene (about 1%) and some beta-pinene and asaronaldehyde (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41330, last accessed 2010-01-29. Use is restricted due to potenial toxicity of beta-asarone (34).

CANANGA ODORATA and Ylang-ylang oil

Ylang-ylang and cananga oils are essential oils that are obtained from two subspecies of the cananga tree (34). In the INCI nomenclature, both are not differentiated.

CANANGA ODORATA *FLOWER EXTRACT*	*CAS* 83863-30-3; EC 281-092-1 (ylang-ylang, ext.) INCI name: CANANGA ODORATA EXTRACT
CANANGA ODORATA *FLOWER OIL*	*CAS 8006-81-3, 68606-83-7; EC / (oils, ylang-ylang) INCI name:* CANANGA ODORATA OIL

Current regulation: …

Clinical data:

Ylang-ylang oil

ISO 4720:2009 nomenclature: *Cananga odorata (Lam.)* Hook. f. et Thomson *forma*

genuina)

In the Larsen 2002 c study, "synthetic ylang-ylang oil" caused 6.4% positive reactions in 218 patients with known contact allergy to fragrance ingredients (1). In a Japanese study, M. Sugawara et al. noted a significant decline of the proportion of patients reacting positively to "ylang-ylang oil 5% pet." from 1971 to 1989, the overall number in patients with cosmetic dermatitis amounting to 176 of 1438 (12.2%, 95% CI: 10.6 – 14.0%) (223). In the Frosch 2002 b study, two fractions of Ylang-Ylang oil (I and II) were separately tested, each at 10% pet. Fraction I yielded 2.6%, fraction II 2.5% positive test reactions (no data on concomitant reactivity given) (17). The deGroot 2000 study, with 1825 consecutively tested patients, found 18 positive PT reactions to "ylang-ylang oil", tested at 4% in pet. (12). The Sugiura 2000 study with 1483 patients with suspected cosmetic dermatitis observed 0.8% positive PT reactions with ylang-ylang oil (5% pet.) (14). The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with ylang-ylang oil (2% pet.) 13.4% positive reactions (9). The Belsito 2006 study (20) yielded 0.6% positive reactions to ylang-ylang oil. The subsequent NACDG 2009 study identified 1.5% positive reactions in 4434 patients PTed with 2% "ylang-ylang oil" (21). The IVDK 2010c study found 2.5% positive reactions in 3175 consecutively tested patients, and 3.9% in 2155 patients tested in the context of a special series (30). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=12 reacted positively to ylang-ylang oil and 3 to "cananga oil" (48).

Cananga oil

ISO 4720:2009 nomenclature: *Cananga odorata (Lam.)* Hook. f. et Thomson *forma macrophylla*. For Oil of cananga (*Cananga odorata (Lam.)* Hook. f. et Thomson, *forma macrophylla*) an ISO standard exists: ISO 3523:2002. Cananga oil is produced by steam distillation of the flowers of *Cananga odorata* (DC.) Hook f. et Thomson subsp. *macrophylla* (*Annonaceae*). The composition resembles that of "ylang-ylang III", but with a higher content of caryophyllene (30-40%). Cananga oil originates almost exclusively in Java; annual production about 50 t. The oil is used mainly in perfuming soaps where it is more stable than ylang-ylang oils due to its lower ester content (34).

Sugiura et al. (2000) found 1.1% positive reactions to "cananga oil", tested 5% pet. (14). Cananga oil (2% pet.) mentioned in the same Portugese study already cited (9) yielded 10.4% positive reactions. In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had positive reactions to cananga odorata oil tested at 2% concentration (13).

Studies with both oils

The Goossens 1997 study found 3 of 111 patients positive to "ylang-ylang oil 5% pet.", and 4 to "cananga oil 15% pet." – all sensitised to other fragrance allergens (23). The Rudzki 1976 study found 1 positive reaction in 200 patients to "cananga" and 4 to "ylang-ylang" essential oil, both tested 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=10 (11.6%) positive reactions to "cananga" and n=8 (9.3%) to "ylang-ylang" essential oils, each tested at 2% pet. (27). Nakayama et al. found 1974 (after (29)) 11 "strong positive" and 15 "weak positive" reactions to "Cananga oil" and 9 and 16, resp., to "Ylang-ylang oil" (unknown test concentration) in 183 patients.

A number of case reports highlight the possibility of occupational contact and sensitisation, e.g. (222, 224).

Additional information:

Ylang-ylang oil

The composition of this essential oil is defined by a standard: ISO 3063:2004. Ylang-ylang oils are obtained by steam distillation of freshly picked blossoms of *Cananga odorata* (DC.) Hook f. et Thomson subsp. *genuina* (*Annonaceae*). The oil is produced mainly in Madagascar and the Comoro islands. Four fractions are collected at progressively longer distillation times and are know as "extra", "I", "II" and "III". The composition of the various oil fractions depends on the duration of distillation. The first fraction has the highest content of strongly odiferous constituents such as p-cresyl methyl ether (5-16%), methyl benzoate (4-9%), (-)-linalool (7-24%), benzyl acetate (5.5-17.5%), and geranyl acetate (2.5-14%). The other fractions contain increasing amounts of sesquiterpene hydrocarbons such as caryophyllene, germacrene-D, and (E,E)-alpha-farnesene (> 70% in "ylang-ylang III"). Components such as p-cresol, eugenol and isoeugenol are important for odour, although they are present only in low concentration (34). According to (30) the maximum observed concentration in ylang-ylang I and II are (in %): germacrene-D (28); (*E*,*E*)-alpha-farnesene (21); caryophyllene (17); *linalool* (I: 19.0; II: 9.5); *benzyl benzoate* (8.0); *farnesol* (4.0); *benzyl salicylate* (4.0); (*E*,*E*)-farnesyl acetate (3.5); *geraniol* (2.5); *isoeugenol* (0.8); *benzyl alcohol* (0.5); *eugenol* (0.5); p-cresyl methyl ether (I: 5.0; II 3.5); methyl benzoate (I: 5.5; II: 3.5); benzyl acetate (I: 10.0; II: 5.0); geranyl acetate (I: 15.0; II: 12.0).

CEDRUS ATLANTICA *BARK OIL*

CAS 92201-55-3; EC 295-985-9 *(Cedrus atlantica, ext. = INCI) / 8000-27-9; EC / (Oils, cedarwood) INCI name: CEDRUS ATLANTICA OIL*

Cedarwood oil

Current regulation: /

Clinical data:

In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=5 (0.7%) positive reactions to cedarwood oil 10% pet. (22). (The exact origin of "cedarwood oil" in this study is not clear.) The IVDK 2010 c study identified 0.8% positive reactions in 6223 patients tested in the context of a special series with a cedarwood oil tagged with CAS # 8000-27-9 (30).

Additional information:

Cedrus Atlantica Bark Oil is the volatile oil obtained from the bark of *Cedrus atlantica, Pinaceae* (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=55309, last accessed 2010-01-05). The main odiferous component is alpha-atlantone [32207-08-2] (39)

Nomenclature also used: Cedrus atlantica wood oil (*Cedrus atlantica* (Endl.) G.Manetti ex Carrière)[22]

See also *Juniperus virginiana*.

[22] ISO 4720:2009 nomenclature

CEDRUS DEODARA *WOOD OIL* — *CAS 91771-47-0; EC* 294-939-5 *(Cedrus deodara, ext.)*

Cedarwood oil

Current regulation: /

Clinical data:

The Rudzki 1976 study found 3 positive reactions in 200 patients to "cedarwood" essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to "Himalayan cedarwood" essential oil 2% pet. (27). (The labelling in the latter report points to *Cedrus deodara* as source of "cedarwood oil" in these 2 Polish studies.)

Additional information:

Cedrus Deodara Wood Oil, Himalayan cedarwood oil (Cedrus deodara (Roxb. ex D.Don) G. Don) [23], is the volatile oil obtained by steam distillation of the stumps of the Deodar Cedar, *Cedrus deodara, Pinaceae* (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=55311, last accessed 2010-01-29).

Several other conifer species are called cedars, and the corresponding oils vary considerably in composition. These include Cedar leaf oil (Thuja oil) produced by steam distillation of fresh leaves and branch ends of *Thuja occidentalis* L. (*Cupressaceae*) from North America, containing a minimum of 60% thujone [8007-20-3] [90131-58-1] (34). Texas cedarwood oil is produced by steam distillation of chopped wood of *Juniperus mexicana* Schiede (*Cupressaceae*), containing alpha-cedrene (15-25%), thujopsene (25-35%), cedrol 20% minimum [8000-27-9] [91722-61-1] (34). Chinese cedarwood oil is similar to Texas cedarwood oil, obtained by steam distillation of *Cupressus funebris* Endl., *Cupressaceae* (*Chamaecyparis funebirs* (Endl.) France), which is a weeping cypress [8000-27-9] [85085-29-6] (34).

CINNAMOMUM CASSIA *LEAF OIL* — *94961-46-6 [invalid] / 8007-80-5; EC / (Oils, cassia) INCI name: CINNAMONUM CASSIA OIL*

Cassia Oil; Cassia leaf Oil; Cinnamon Oil Chinense

CINNAMOMUM ZEYLANICUM *BARK OIL* — *CAS 84649-98-9; EC* 284-635-0 *(Cinnamonum zeylanicum, ext. = INCI)*

Cimmamon Bark Oil Ceylon; Cinnamon Oil Ceylon

Current regulation: /

Clinical data:

The Rudzki 1976 study found 2 positive reactions in 200 patients to "cassia" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=24

[23] ISO 4720:2009 nomenclature

(27.9%) positive reactions to “cassia” essential oil 2% pet. (27).

A 32 year old Spanish physiotherapists developed vesicular hand dermatitis after using a "balsam from ash extract" cream. PTing revealed positive reactions to this cream, the FM I, eugenol, and 2 components of the cream: "cinnamon oil" (0.5% pet.) and clove oil (1% pet.) (225).

Additional information:

ISO 4720:2009 nomenclature: *Cinnamomum tsumu* Helms, syn. *Cinnamomum cassia* auct. and *Cinnamomum zeylanicum* Blume syn. *Cinnamomum verum* J. Presl, respectively. Cassia oil (Chinese cinnamon oil) is obtained by steam distillation of the leaves, twigs, and bark of *Cinnamonum aromaticum* Nees (*C. cassia* Blume, *Lauraceae*). In contrast to cinnamonum bark oil (see below), cassia oil contains a considerable amount of 2-methoxycinnamal (3-15%), in addition to its main constituent, cinnamal (70-88%). Cassia oil is predominantly used in flavouring soft drinks, with an annual production of a few hundred tons (34). For Oil of cassia, Chinese type (Cinnamomum aromaticum Nees, syn. Cinnamomum cassia Nees ex Blume) an ISO standard exists: ISO 3216:1997

Cinnamomum Zeylanicum Bark Oil is the volatile oil expressed from the bark of the Ceylon Cinnamon, Cinnamomum zeylanicum, Lauraceae. It contains mainly cinnamaldehyde (34), e.g. 50-60%, and lesser quantities of eugenol (4-8%), phellandrene (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=75370, last accessed 2009-11-16). For Oil of cinnamon leaf, Sri Lanka type (Cinnamomum zeylanicum Blume) an ISO standard exists: ISO 3524:2003

Cinnamomum Cassia Leaf Oil is the volatile oil obtained by steam distillation from the leaves and twigs of the Chinese Cinnamom, Cinnamomum cassia (L.), Lauraceae. It contains 80% eugenol (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=75368, last accessed 2009-11-16). The cinnamon leaf oil produced by steam distillation of the leaves of Cinnamonum zeylanicum Blume (C. verum J.S. Presl) similarly has a content of 70-83% eugenol (34).

Considering the content of well-known allergenic compounds, the essential oil is considered an Established contact allergen in humans,

CITRUS AURANTIUM AMARA *FLOWER OIL*	*CAS 8016-38-4, 68916-04-1; EC / (Oils, neroli) /*
Neroli oil	
CITRUS AURANTIUM AMARA *PEEL OIL EXPRESSED*	*72968-50-4; EC* 277-143-2 *(Orange, sour, ext.)*
"Bitter Orange Oil"	
	INCI names: CITRUS AURANTIUM AMARA …

Current regulation: /

Clinical data:

The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "neroli oil" (2% pet.) 6.6% positive reactions (9). The Rudzki 1976 study found 3 positive reactions in 200 patients to “bitter orange” essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=2 (2.3%)

positive reactions to “bitter orange” essential oil 2% pet. (27). The IVDK 2010 c study identified 0.7% positive reactions in 6220 patients tested in the context of a special series (30)

Additional information:

ISO 4720:2009 nomenclature: *Citrus aurantium* L., syn. *Citrus amara* Link, syn. *Citrus bigaradia* Loisel, syn. *Citrus vulgaris* Risso. For Oil of neroli (Citrus aurantium L. spp. aurantium, syn. Citrus aurantium L. spp. amara var. pumilia) an ISO standard exists: ISO 3517:2002. Citrus Aurantium Peel Oil Expressed is an essential oil expressed from the fresh epicarps of the Sour Orange, Citrus aurantium, Rutaceae. It contains D-limonene (about 90%), citral, decanaldehyde, methyl anthranilate, linalool, terpineol (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41394, last accessed 2010-01-29. The aldehyde content is lower and the ester content (e.g., linalyl and geranyl acetate) is higher than in sweet orange oil (34). It is predominantly used for flavouring alcoholic beverages. According to (30) the maximum observed concentration in neroli oil are (in %): linalool (44); limonene (18); β-pinene (17); linalyl acetate (15); *trans*-β-ocimene (8); geranyl acetate (5); *trans*-nerolidol (5); (*E*,*E*)-farnesol (4); myrcene (4); farnesol (4,0); geraniol (3,5); citral (0,3) (30).

CITRUS AURANTIUM AMARA *LEAF OIL* *72968-50-4; EC* 277-143-2 *(Orange, sour, ext.)*

Petitgrain oil Paraguay / … bigarade

Current regulation: /

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to “Petitgrain bigarade” and “Petitgrain Paraguay” essential oil each, both tested at 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=7 (8.1%) positive reactions to “Petitgrain bigarade” and n=4 (4.6%) to “Petitgrain Paraguay” essential oil each, both tested at 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: Citrus sinensis L. Pers. X Citrus aurantium L. ssp. amara var. pumilia. Petitgrain oils in general are steam distilled from the leaves of citrus trees. Citrus Aurantium Leaf Oil is an essential oil obtained from the leaves of the Sour Orange, Citrus aurantium, Rutaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41392, last accessed 2010-02-10). Petitgrain oil Paraguay is obtained from an acclimatised variety of the bitter orange tree. Main constituents are linalool (15-30%) and linalyl acetate (40-60%). A number of trace constituents contribute essentially to the odour (34). Petitgrain oil bigarade is derived from the same species of tree grown in France, Italy, Spain and North Africa (34). For Oil of bitter orange petitgrain, cultivated (Citrus aurantium L.) an ISO standard exists: ISO 8901:2003.

Considering the content of well-known allergenic compounds, the essential oil is regarded as an established contact allergen in humans

CITRUS BERGAMIA *PEEL OIL EXPRESSED* *CAS 89957-91-5, 8007-75-8; EC*

	289-612-9 *(Bergamot, ext.)*
Bergamot Oil, Bergamot Orange Oil	*INCI: CITRUS AURANTIUM BERGAMIA EXTRACT*

Current regulation: /

Clinical data:

The Rudzki 1976 study found 3 positive reactions in 200 patients to “Bergamot” essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found no positive reaction to “Bergamot” essential oil 2% pet. (27). In 63 patients positive to the FM I, 2 had a positive PT reaction to bergamot oil, 2% pet., in the Santucci 1987 study (28). A case report from Zacher and Ippen describes 2 patients with allergic contact dermatitis due to bergamot oil (191), one a worker in a perfume factory, the other sensitised by non-occupational use of cosmetics.

Additional information:

ISO 4720:2009 nomenclature: *Citrus bergamia* (Risso et Poit.), syn. *Citrus aurantium* L. subsp. *bergamia* (Wight et Arnott) Engler. Citrus Bergamia Peel Oil Expressed is an essential oil expressed from the epicarps of the Bergamot, Citrus bergamia risso, Rutaceae. It contains 35-45% L-linalyl acetate, about 6% linalool, D-limonene, DL-limonene and bergaptene (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41398, last accessed 2009-11-27). According to Surburg/Panten: linalyl acetate 22-36%, linalool 3-15%, geranial 0.25-0.5%, citral 1%, with a relatively low terpene content of 25-50% (34, 39). Bergaptene content by HPLC is 0.18-0.38% (34). Annual production from Italy, Brazil, Spain and Ivory Coast is 100 to 150 t. For Oil of bergamot [Citrus aurantium L. subsp. bergamia (Wight et Arnott) Engler], Italian type an ISO standard exists: ISO 3520:1998.

***CITRUS LIMONUM** PEEL OIL EXPRESSED*	*CAS 84929-31-7, 8008-56-8; EC* 284-515-8 *(Lemon, ext.)*
Lemon oil	INCI names: CITRUS MEDICA LIMONUM …

Current regulation: /

Clinical data:

The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "lemon oil" (2% pet.) 4.5% positive reactions (9). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=2 (0.3%) positive reactions to “lemon oil” 2% pet. (22).

The Rudzki 1976 study found 1 positive reaction in 200 patients to “lemon” essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=2 (2.3%) positive reactions to “lemon” essential oil 2% pet. (27). The IVDK 2010 c study identified 0.3% positive reactions in 6467 patients tested in the context of a special series (30).

Additional information:

ISO 4720:2009 nomenclature: *Citrus limon* (L.) Burm. f. According to (30) the maximum observed concentration in lemon oil are (in %): limonene (80); ß-pinene (16.5); γ-terpinene (12); citral (3.0); geranial (2.0); neral (1.2); β-bisabolene (0.9); geranyl

acetate (0.7); neryl acetate (0.6); linalool (0,3); geraniol (0,2) (30). An ISO standard exists for Oil of lemon [Citrus limon (L.) Burm. f.], obtained by expression: ISO 855:2003. The composition of lemon oil depends on the variety of lemon an the country of origin, see table from (34).

Table 3. Specifications for qualities of lemon oils of different origins

Parameter	Type		Mediterranean		Equatorial
	American Origin				
	Coast	Desert	Italy	Spain	Ivory coast, Brazil
d_{20}^{20}	0.851–0.857	0.849–0.854	0.850–0.858	0.849–0.858	0.845–0.854
n_D^{20}	1.4370–1.4760	1.4370–1.4760	1.4370–1.4760	1.4370–1.4760	1.4370–1.4790
α_D^{20}	+57° to +65°6′	+67° to +78°	+57° to +66°	+57° to +66°	+57° to +70°
Composition by GC [area %]					
β-Pinene	9–14	10–13	10–16.5	10–16.5	7–16
Limonene	63–70	70–80	60–68	60–70	59–75
γ-Terpinene	8.3–9.5	6.5–8	8–12	8–128–12	6–12
Neral	0.6–0.9	0.3–0.6	0.6–1.2	0.4–1	0.2–1.2
Geranial	1.0–2	0.5–0.9	0.8–2	0.6–2	0.5–2
Evaporation residue [weight %]					
	1.75–3.9		1.5–3.9	1.5–3.9	1.5–4
Carbonyl value					
	8–14	6.25–12	11–17	11–17	6–17
CD value	min. 0.2	min. 0.2	0.45–0.9	0.4–0.9	0.2–0.96

CITRUS PARADISI *PEEL OIL*

Grapefruit oil, expressed

CAS 8016-20-4 ; EC /

INCI: CITRUS GRANDIS OIL

Current regulation: II/358 R1

Clinical data: /

Additional information:

Citrus Paradisi Peel Oil is the volatile oil expressed from the peel of the Grapefruit, Citrus paradisi, Rutaceae http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=55434

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

CITRUS SINENSIS *(syn.: AURANTIUM DULCIS) PEEL OIL EXPRESSED*

CAS 97766-30-8, 8008-57-9, EC 307-891-8 *(Orange, sweet,*

	Valencia, ext. = INCI) / 8028-48-6; EC 232-433-8 *(Orange, sweet, ext.)*
(Sweet) Orange oil	INCI names: CITRUS AURANTIUM DULCIS …

Current regulation: /

Clinical data:

The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "orange oil" (2% pet.) 4.5% positive reactions (9). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=1 (0.1%) positive reactions to orange oil 2% pet. (22). The Rudzki 1976 study found 1 positive reaction in 200 patients to “sweet orange” essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to “sweet orange” essential oil 2% pet. (27). In the Frosch 1995 dose-finding pilot study, neither positive nor irritant reaction to 1% and 5% “orange oil Bras.” in pet., tested in 205 consecutive patients in Dortmund and Göttingen, were observed (15). The IVDK 2010 c study identified 0.2% positive reactions in 6246 patients tested in the context of a special series (30).

Additional information:

ISO 4720:2009 nomenclature: *Citrus sinensis* (L.) Osbeck. For Oil of sweet orange (Citrus sinensis (L.) Osbeck), CAS 8008-57-9, obtained by mechanical treatment, an ISO norm exists: ISO 3140:2005. The oils have a high terpene hydrocarbon content (> 90%, mainly (+)-limonene. Important for aroma are aldehydes, mainly decanal and citral, and aliphatic and terpenoid esters. The sesquiterpene aldehydes alpha-sinensal [17909-77-2] and beta-sinensal [6066-88-8] contribute particularly to the special sweet aroma (34). According to (30) the maximum observed concentration in sweet orange oil are (in %): *limonene* (95.0); *linalool* (0.7); n-decanal (0.7); *citral* (0.3); alpha-sinensal (0.05); beta-sinensal (0.06) (30). Worldwide production is more than 30000 tons / year. Main uses comprise the flavouring of beverages and confectioneries and perfuming E.d.C, soaps and household products.

For the latter uses relevant here, both “Orange peel oil, sweet (Citrus sinensis (L.) Osbeck) (8008-57-9)“, “Orange peel, sweet, extract (Citrus sinensis L. Osbeck) (8028-48-6)“ and “Orange, sweet, Valencia, ext. (97766-30-8)” are among the top 100 used fragrance materials and classified as R43 (IFRA, pers. comm. 2010).

ORANGE OIL TERPENES (CAS # 68647-72-3) are a “top 100 mixture of substances and classified as R43 (IFRA, pers. comm.2010). Other names: ORANGE, SWEET, TERPENES (REACH); Terpenes and Terpenoids, sweet orange-oil (REACH). The CAS entry refers to a group of substances “Terpenes and Terpenoids, sweet orange-oil“ (REACH).

CITRUS TANGERINA *…*	*CAS 223748-44-5; EC /*
Oil of tangerine	[no info in CAS database]

Current regulation: /

Clinical data:

In a 17 year old girl, the perfume used for 3 months caused ACD due to the ingredient “oil of tangerine”, with a strong positive PT reaction (to 2% or 10% in pet.; 50 controls

negative) (226).

Additional information:

Citrus Tangerina Peel Oil is the volatile oil expressed from the peel of the ripe fruit the Tangerine, *Citrus Tangerina, Rutaceae* (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=55441, last accessed 2010-01-29); (*Citrus tangerina* Tanaka).

***CORIANDRUM SATIVUM** HERB OIL*

Coriander oil

CAS 84775-50-8; EC 283-880-0 *(Coriander, ext.)*

INCI: CORIANDRUM SATIVUM EXTRACT

Current regulation: /

Clinical data:

The Rudzki 1976 study found 2 positive reactions in 200 patients to "coriander" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to "coriander" essential oil 2% pet. (27).

Additional information:

Coriander Sativum Herb Oil is an essential oil obtained from the herbs of the Coriander, Coriandrum sativum L., Umbelliferae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39388, last accessed 2010-01-29). The main component of coriander oil is linalool (by GC: 65-78%) and mono- and polyunsaturated fatty aldehydes contributing to the particular aroma. In contrast to the seed oil, coriander leaf oil contains these aldehydes as main constituents, e.g. 2-deccanal and 2-dodecanal (34). For Oil of coriander fruits (Coriandrum sativum L.) an ISO standard exists: ISO 3516:1997.

***CYMBOPOGON** OILS*

Cymbopogon oils are produced from several aromatic grasses that belong to the genus *Cymbopogon* Speng. (*Poaceae*). The oils are obtained by steam distillation of the aerial parts of the plants (34).

The composition of the essential oil derived from *Cymbopogon flexuosus* (Nees ex Steudel) J.F. Watson is defined by a standard: ISO 4718:2004, as is the oil derived from *Cymbopogon citratus:* 3217:1974.

***CYMBOPOGON CITRATUS** LEAF OIL*

Cymbopogon citratus (DC.) Stapf.[24]

CAS 89998-14-1; EC 289-752-0 *(Cymbopogon citratus, ext. = INCI)*

[24] ISO 4720:2009 nomenclature

Lemon Grass Oil; Indian Verbena Oil; Indian Melissa Oil

***CYMBOPOGON SCHOENANTHUS** OIL*

Cymbopogon flexuosus (Nees ex Steudel) J.F. Watson[25]

CAS 8007-02-1; EC 289-754-1 *(oils, lemongrass) / 89998-16-3; EC* 289-752-0 *(Cymbopogon Schoenanthus, ext. = INCI)*

Lemon Grass Oil

Current regulation: /

Clinical data:

The Frosch 2002 b study on 1606 consecutive patients reported 1.6% positive reactions to "lemongrass oil (East India), CAS 8007-02-1", PTed at 2% pet. (17). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 3 positive reactions to lemongrass oil were observed (3). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=6 (0.8%) positive reactions to lemongrass oil 2% pet. (22). The IVDK 2010 c study identified 0.6% positive reactions in 2435 consecutively tested patients and 2.3% positive reactions in 8445 patients tested in the context of a special series (30).

Additional information:

Cymbopogon Citratus Leaf Oil is an essential oil obtained from the leaves of the Lemon Grass, *Cymbopogon citratus* (DC., ex Nees), *Poaceae*. It contains citral (75-85%), methylheptenone, citronellal, geraniol, limonene (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39457, last accessed 2009-11-12). According to Surburg/Panten, by GC: neral (31-40%), geranial (40-50%) (34).

Indian lemongrass oil is obtained by the so-called Indian variety of lemongrass, *Cymbopogon flexosus* (Nees ex Steud.) Stapf. Content by GC: 25-35% neral, 35-47% geranial (34).

Cymbopogon Schoenanthus Oil is the volatile oil obtained by the steam distillation of fresh Lemon Grass, *Cymbopogon schoenanthus* (L.), *Poaceae* (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=75419, last accessed 2009-11-12). According to (30) the maximum observed concentration in lemongrass oil are (in %):*citral* (85.0); *geraniol* (7.0); *limonene* (4.0); geranyl acetate (2.2); caryophyllene (1.6); trans-isocitral (1.4); 6-methyl 5-hepten-2-one (1.3); caryophyllene oxide (1.2); 4-nonanone (1); *citronellol* (0.8); *eugenol* (0.3); *linalool* (0.2) (also according to (227))

In a LLNA study by RIFM, the lemongrass oil as used was reported to contain 68.8% citral, 6.7% limonene, 6.1% geraniol, 2.2% geranyl acetate, 1.6% caryophyllene, 1.4% trans-isocitral, 1.3% 6-methyl 5-hepten-2-one, 1.2% caryophyllene oxide and 1% 4-nonanone, according to analyses of the supplier. The EC3 value was calculated to be 6.5% (227).

***CYMBOPOGON MARTINI** HERB EXTRACT*

CAS 84649-81-0; EC 283-461-2 *(Cymbopogon Martini, ext)*

[25] ISO 4720:2009 nomenclature

INCI: CYMBOPOGON MARTINI OIL

Palmarosa oil

Current regulation: /

Clinical data: /

Additional information:

ISO 4720:2009 nomenclature: *Cymbopogon martini* (Roxb.) Will. Watson *var. motia* and var. *sofia*. Cymbopogon Martini Herb Extract is an extract obtained from the herbs of the plant, *Cymbopogon martini*, Gramineae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39460, last accessed 2009-11-24), namely, by steam distillation of wild or cultivated Cymbopogon martini (Roxb.) J.F. Wats., collected when in blossom (34).The main constituent is geraniol (72-94%) (34).

In a LLNA study by RIFM, the palmarosa oil as used was reported to contain 79.4% geraniol, 9.4% geranyl acetate and 1.9% caryophyllene, according to analyses of the supplier. The EC3 value was calculated to be 9.6% (227).

CYMBOPOGON NARDUS *HERB OIL* — *CAS 89998-15-2; EC* 289-753-6 *(Cymbopogon nardus, ext. = INCI)*

Citronella Oil (Sri Lanka)

CYMBOPOGON WINTERIANUS *HERB OIL* — *CAS 91771-61-8; EC* 294-954-7 *(Cymbopogon Winterianus, ext. = INCI)*

Citronella Oil (Java)

Current regulation: …

Clinical data:

The Rudzki 1976 study found 5 positive reactions in 200 patients to “citronella” essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=1 (1.1%) positive reactions to “citronella” essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Cymbopogon nardus* (L.) W. Watson *var. lenabatu* Stapf. and *Cymbopogon winterianus* Jowitt, respectively. Cymbopogon Nardus Herb Oil is an essential oil obtained from the herbs of the plant, Cymbopogon (syn: Andropogon) nardus (L.), Gramineae. The Ceylon citronella oil contains geraniol (about 60%), citronellal (about 15%), camphene, limonene, linalool, borneol. According to Surburg/Panten, the Sri Lankan oil contains citronellal (3-6%), borneol (4-7%), citronellol (3-8.5%), geraniol 15-23%) and methyl isoeugenol (7.11%) (34).

The Java citronella oil contains 25-50% citronellal, 25-45% geraniol (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39469, last accessed 2009-11-24). Cymbopogon Winterianus Herb Oil as a synonym for Java citronella oil is obtained from the herbs of the plant, Cymbopogon winterianus,
Gramineae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=sea

rch.details&id=39472, last accessed 2009-11-24). This oil, produced in Taiwan and Java, contains citronellal (31-40%), geraniol (20-25%), citronellol (8.5-14%), geranyl acetate (2.5-5.5%), citronellyl acetate (2-4%) and many minor components. Annual worldwide production is currently at around 1000 t (34). For Oil of citronella, Sri Lankan type (Cymbopogon nardus (L.) W. Watson var. lenabatu Stapf.) an ISO standard exists: ISO 3849:2003, for Oil of citronella, Java type the ISO 3848:2001.

In a LLNA study by RIFM, the citronella oil as used was reported to contain 36.6% citronellal, 20.6% geraniol, 4.1% limonene, 3.7% geranyl acetate, 3.0% citronellyl acetate, 2.6% elemol, 2.2% beta-bourbonene, 1.9% delta-cadiene, 1.6% isopugenol I, 1.4% germacrene D and eugenol and linallol at < 1%, according to analyses of the supplier. The EC3 value was calculated as > 50 % (227).

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

EUCALYPTUS SPP. *LEAF OIL* — *CAS 92502-70-0; EC* 296-357-7 *(Eucalyptus, ext. = INCI)*

Eucalyptus *Oil* — *CAS 8000-48-4; EC / (Oils, eucalyptus) INCI: EUCALYPTUS GLOBULUS OIL*

Current regulation: /

Clinical data:

In a study with 218 fragrance sensitive patients, 1.8% reacted positively to 10% eucalyptus oil (pet.) (1). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 1 positive reaction to "eucalyptus oil" was observed (3). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=4 (0.6%) positive reactions to eucalyptus oil 2% pet. (22). The Rudzki 1976 study found 3 positive reactions in 200 patients to “eucalyptus” essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=1 (1.1%) positive reactions to “Eucalyptus” essential oil 2% pet. (27). The IVDK 2010 c study identified 0.2% positive reactions in 6680 patients tested in the context of a special series (30).

In a professional athlete, the use of an "analgesic and anti-inflammatory cream" over 2 years lead to ACD, which was attributed to eucalyptol (eucalyptus oil, 1% pet., 25 controls negative), the sole ingredient of the cream eliciting a positive PT reaction (228)

Additional information:

ISO 4720:2009 nomenclature: *Eucalyptus globulus* Labill. Eucalyptus oils are produced from plants belonging to the genus *Eucalyptus* (*Myrtaceae*), which includes about 500 species in Australia, the country of origin, alone. At present, few of the oils, which are used to characterise species, are commercially important (34). Some species are rich in 1,8-cineole (80-85% content). Other species contain less cineole, but 10-22% alpha-pinene. *E. citriodora* predominantly contains citronellal (min. 75% by GC), with some citronellol and isopulegol (5-10% each) (34). *E. dives* contains (-)-piperitone and 15-25% alpha-phellandrene (34). According to (30) the maximum observed concentration in eucalyptus oil are (in %): 1,8-cineole (58; 70-80 after rectification); α-pinene (22); limonene (8); para-cymene (5); trans-pinocarveol (5); aromadendrene (10); globulol (2.5) [the latter 2 components only traces after rectification] (30).

For Crude or rectified oils of Eucalyptus globulus (Eucalyptus globulus Labill.) an ISO standard exists**:** ISO 770:2002.

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

EUGENIA CARYOPHYLLUS *LEAF / FLOWER OIL* — *CAS 8000-34-8; EC / (Oils, clove)*

Clove oil — INCI: EUGENIA CARYOPHYLLUS OIL

Current regulation: /

Clinical data:

In the Larsen 2002 c study, 19.3% of patients with known contact allergy to fragrance ingredients reacted positively to "clove bud oil" (10 % pet.) (1). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 2 positive reactions to "clove oil" were observed (3). The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with clove oil (2% pet.) 13.4% positive reactions (9). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 1.6% positive reactions 2% pet. (22). The Rudzki 1976 study found 2 positive reactions in 200 patients to "clove" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=12 (13.3%) positive reactions to "clove" essential oil 2% pet. (27). The IVDK 2010 c study identified 1.5% positive reactions 6893 patients tested in the context of a special series (30).

A 32 year old Spanish physiotherapists developed vesicular hand dermatitis after using a "balsam from ash extract" cream. PTing revealed positive reactions to this cream, the FM I, eugenol, and 2 components of the cream: cinnamon oil (0.5% pet.) and clove oil (1% pet.) (225).

Additional information:

ISO 4720:2009 nomenclature: *Syzygium aromaticum* (L.) Merr. & L. M. Perry syn. *Eugenia caryophyllus* (Spreng.) Bullock & S. G. Harrison. Standards regarding the composition of clove oil are available: ISO 3141:1997, ISO 3142:1997, ISO 3143:1997. Clove oils are produced from the clove tree *Szyigium aromaticum* (L.) Merr. et L.M. Perry [*Eugenia caryophyllus* (Speng.) Bullock ex S.G. Harrison. The content of clove bud, clove leaf and clove stem oil has, with little variation, been determined by GC as 75-92% eugenol, 2-17% caryophyllene and 0.2-15% eugenyl acetate – the latter compound found in particularly high concentration in bud oil (34). According to another source, the following maximum content (%) has been observed regarding the constituents listed: eugenol (92,0);

caryophyllene (17); eugenyl acetate (15); isoeugenol (0.5) (30).

In a LLNA study by RIFM, the clove leaf oil as used was reported to contain 85.3% eugenol, 9.9% caryophyllene and 2.2% alpha humulene, according to analyses of the supplier. The EC3 value was calculated to be 7.1% (227).

EVERNIA FURFURACEA *LICHEN EXTRACT* *CAS 90028-67-4; EC* 289-860-8 *(Evernia furfuracea, ext. = INCI)*

Tree moss extract

Current regulation: /

Clinical data:

The Larsen 1977 study in 20 “perfume-sensitive patients” yielded n=6 positive reactions with “treemoss abs. in benzyl benzoate, 5% petrolatum” (18). In the IVDK 2007 study, 2.7% (95% CI: 2.0 – 3.6%) of 1658 consecutive patients had a positive reaction to “tree moss absolute” (4). In the Groningen 2009 study, 2.5% (95% CI: 1.1 – 4.9%) had positive reactions to the allergen, tested at 2%., i.e., twice the commonly used concentration, and not in pet., but in diethylphthalate (6). The IVDK 2010 study, 6.02% (95% CI: 4.90 – 7.14%; percentages standardised for age and sex) of 1947 patients PTed reacted to the compound (7).

Additional information:

Syn.: *Pseudoevernia furfuracea* (L.) Zopf (53). The lichen grows on the bark of pine and fir trees. The extraction process with carbohydrate solvents yields a “concrete” (2-5% yield) which, in a next step eliminating waxy compounds, is extracted with warm alcohol and subsequent cooling, yielding an “absolute” (40-60% yield) (53).

EVERNIA PRUNASTRI *CAS 90028-68-5; EC* 289-861-3 *(Evernia prunastri, ext. = INCI)*

Oak moss abs.

Current regulation: Annex III, part 1, n° 91

Clinical data:
In the “background information” section of the 1999 opinion, oak moss exctract is classified as “most frequently reported allergen”; in consecutive PT patients, about 2.8% positive reactions had been reported (33). ´The German MAK commission has labelled oak moss extract as ‘sensitising to the skin’ (229).

Since the last SCCNFP-opinion of 1999, a “polymer based method” was developed to reduce the natural content of these two compounds from around 1 - several percent to < 75 ppm for atranol and < 25 ppm for chloratranol. However, PTing 14 subjects with previous positive PT reactions to the “oak moss” allergen preparation with the modified Evernia prunastri material still elicited positive reactions in 8/14 subjects; thus, the reduction in allergen content was deemed unsafe for the consumer (230). In a study of 885 consecutive eczema patients tested in Gentofte, Denmark, 3.2% had a positive or follicular patch test response to oak moss absolute. Two types of oak moss absolute were tested, one contaminated by resin acids and one without any detectable resin acids. There was no difference in reactivity between the two types of oak moss absolute

(231). The IVDK 2007 study yielded 2.2% (95% CI: 1.6 – 3-0%) positive reactions in 2063 consecutively tested patients (4). In the Groningen 2009 study, 1.9% (95% CI: 0.7 – 4.0%) had positive reactions to oak moss, tested at 2% pet., i.e., twice the commonly used concentration (6). In the An 2005 study, 6 of 422 consecutive patients, i.e., 1.4%, had positive reaction (13) (test concentration 2% pet.). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 5.0% positive reactions (22). The IVDK 2010 study, 1.81% (95% CI: 1.07 – 2.56%) of 1213 consecutively tested patients reacted to the compound, while 5.59% (95% CI: 4.90 – 6.27%) of 4482 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were tested with *E. prunastri* extract, yielding 2 positive reactions (48).

L. Kanerva et al. report on a 41 year old female hairdresser in whom oak moss abs. contained in a perming solution (concentration in the product unknown) was unequivocally identified as allergen causing (i) occupational hand dermatitis and (ii) scalp dermatitis after application to the own hair (232). Another case of occupational hand dermatitis in a grinding engineer was, at least partly, attributable to contact sensitisation to "oak moss resin" contained in a soluble oil (233).

Additional information:

Source: Evernia prunastri (Oak moss) (*Evernia prunastri* var. *prunastri* L. Ach). Oak moss is extracted as described above. Chloratranol and atranol are the degradation products of chloratranorin and atranorin, resp., which are recognised as the main sensitisers in *Evernia prunastri* extracts.

ILLICIUM VERUM *FRUIT OIL*

"Anise Oil", Star anise oil

CAS 84650-59-9, 8007-70-3; EC 283-518-1

(Star anise, Illicium verum, ext. = INCI)

Current regulation: /

Clinical data:

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

Additional information:

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

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

Current regulation: /

Clinical data:

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

Additional information:

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

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

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

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

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

JUNIPERUS VIRGINIANA *OIL*

CAS 8000-27-9; EC / (Oils, cedarwood) [this also refers to Cedrus atlantica …) / 85085-41-2; EC 285-370-3 *(Juniper, Juniperus virginiana, ext. = INCI)*

JUNIPERUS VIRGINIANA *WOOD OIL*

CAS 85085-41-2; EC 285-370-3

Cedar Wood Oil (Virginian)

Current regulation: /

Clinical data:

In the Frosch 2002 b study, a total of 0.6% of 1606 consecutive patients had a positive PT to “cedarwood oil (Moroccan and Chinese 1:1)”, tested 10% in pet. (17). After application of Penaten-baby™ oil as immersion oil for dermatoscopy a patient developed multiple patches of eczema at the application sites. Investigation revealed that the oil was kept in a bottle previously used for *Juniperus virginiana* oil, to which contact sensitisation was verified by patch testing (235).

Additional information:

ISO 4720:2009 nomenclature: *Juniperus virginiana* L.. Juniperus Virginiana Oil is the volatile oil obtained from the fruits and leaves of the Red Cedar, Juniperus virginiana L., Cupressaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=78070, last accessed 2010-01-05)

Juniperus Virginiana Wood Oil is an essential oil obtained from the wood and twigs of the Red Cedar, Juniperus virginiana L., Cupressaceae. It contains chiefly (alpha and beta) cedrene and cedral (cedar camphor), cuparene, thujopsene, widdrol (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39767, last accessed 2009-11-12)(235). According to Surburg/Panten by GC: alpha-cedrene 22-35%, thujopsene 10-25%, cedrol 16-25% (34).

See also *Cedrus atlantica*. According to (30) the maximum observed concentration in cedar wood oil are (in %): α-cedrene (32); thujopsene (25); cedrol (25); β-cedrene (6); widdrol (5) and cuparene (traces) (30).

For Oil of cedarwood, Virginian (Juniperus virginiana L.) an ISO standard is available: ISO 4724:2004. For Oil of cedarwood, Texas (Juniperus mexicana Schiede) an ISO standard exists: ISO 4725:2004.

LAURUS NOBILIS *OIL*

CAS 8002-41-3; EC / (Oils, laurel) INCI: LAURUS NOBILIS OIL / 8007-48-5; EC / (Oils, sweet bay)/ 84603-73-6; EC 283-272-5 *(Laurus nobilis, ext.) INCI: LAURUS NOBILIS EXTRACT*

Laurel oil

Current regulation: Annex II, n° 359 (seed oil)

Clinical data:

In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=4 (0.6%) positive reactions to “laurel oil” 2% pet. (22).

After sensitisation by a one-time occlusive application a 36 year old Turkish patient developed widespread allergic contact dermatitis 3 days after massage with olive oil containing Laurus nobilis oil; sensitisation was proven by a strong positive reaction to the commercial test preparation and the massage oil previously used (236). Topical application of laurel oil for knee arthropathy led to an erythema exudativum multiforme-like rash on the legs of a 63 year old patient; interestingly, laurel oil yielded a “target like” strongly positive PT reaction in this case (237). In an earlier Turkish case with a similar history, the EEM-like appearance was lacking; however, a very intense, edematous reaction was noted (238). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 2 positive reactions to "laurel oil" were observed (3). The IVDK 2010 c study identified 1.0% positive reactions in 6297 patients tested in the context of a special series (30).

Additional information:

ISO 4720:2009 nomenclature: *Laurus nobilis* L. Laurel leaf oil is obtained by steam distillation of leaves from Laurus nobilis L. (Lauraceae), an evergreen cultivated primarily in the Mediterranean countries. The main components are 1,8-cineole (30-70%), linalool (about 10%) and eugenol (34). According to (30) the maximum observed concentration in laurel oil are (in %): 1,8-cineole (70); ß-caryophyllene (11); *linalool* (11); *limonene* (5.0); *eugenol* (2.0); *geraniol* (0.3) (30).

LAVANDULA HYBRIDA *HERB OIL*

CAS 91722-69-9; EC 294-470-6 *(Lavender, Lavandula hybrida, ext. = INCI)*

Lavandin Oil

Current regulation: /

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to “lavandin” essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=4 (4.6%) positive reactions to “lavandin” essential oil 2% pet. (27). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% lavandin oil in pet., tested in 205 consecutive patients in Dortmund and Göttingen, and just 1 irritant reaction to

the higher concentration, were observed (15).

Additional information:

ISO 4720:2009 nomenclature: *Lavandula angustifolia* Mill. x *Lavandula latifolia* Medik. Lavandula Hybrida Herb Oil is an essential oil distilled from the flowering herbs of the Lavendin, *Lavandula hybrida, Labiatae* (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39789, last accessed 2010-01-29. Nomenclature according to Surburg/Panten: *Lavandula* x *intermedia* Lois, which is a hybrid of lavender and spike (see below) (34). The oils from the most important variants, abrial and grosso, contain linalool (24-38%), linalyl acetate (20-38%) as well as 1,8-cineole (4-11%), and camphor (6-11%) (34). A third variant is called super because of its high concentration of linalyl acetate (35-47%), more closely resembling lavender oil (34). For Oil of lavandin Grosso (Lavandula angustifolia Mill. x Lavandula latifolia Medik.), French type an ISO standard exists: ISO 8902:2009, for Oil of lavandin Abrial (Lavandula angustifolia Miller x Lavandula latifolia Medikus), French type a different ISO standard: ISO 3054:2001.

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

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

LAVANDULA OFFICINALIS *FLOWER OIL*

CAS 84776-65-8, 8000-28-0; EC 283-994-0 *(Lavender, Lavandula angustifolia angustifolia, ext. = INCI)*

Lavender oil

Current regulation: /

Clinical data:

In a large series from Nagoya, Japan, 1483 patients were tested with lavender oil 20% in pet., with overall 3.7% positive reactions from 1990 to 1998. However, within this period, a sharp increase was noted in 1997 and 1998, which as attributed to changed exposure by M. Sugiura et al. (14). On the individual level, relevance of positive reactions remained unclear in about half of the cases. The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "lavender absolute" (2% pet.) 6.6% positive reactions (9). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had positive reactions to “Lavendula augustifolia oil” (Lavender absolute) 2% (13). The Goossens 1997 study found 4 of 111 patients positive to “lavender oil 20% pet.”)– all of them sensitised to other fragrance allergens (23). The Rudzki 1976 study found no positive reaction in 200 patients to “lavender” essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to “lavender” essential oil 2% pet. (27). Nakayama et al. found 1974 (after (29)) 6 “strong positive” reactions to “Lavender oil” (unknown test concentration) in 183 patients. In a study from Alicante, Spain, 86 selected patients were tested with “lavender absolute”, yielding 2 positive reactions (48).

R. Goiriz et al. report on a case of photo contact allergy (10 controls negative) in a 45 year old woman developing after application of a ketoprofen-containing topical gel (“Fastum”)(239). A physiotherapist developed acute, recurrent dermatitis after use of “Difflam® gel”, scented with lavender oil. Both the gel and lavender oil (2% pet.) tested positive; avoidance resulted in clearing (240). In a study on 218 patients with known

contact allergy to fragrance ingredients, Larsen (2002 c) found positive reactions to 10% lavender oil (pet.) in 2.8% of these (1). A case of vulvovaginitis with spread and affecting the dominant hand applying various tea tree and lavender oil creams was reported by S. Varma; the PT with 10% lavender oil abs. in pet. (50 controls negative) was positive (241). In two cases, facial "pillow dermatitis" due to lavender oil, applied to the pillows, developed, confirmed by positive PT to lavender abs. (2% pet.) (242).

Additional information:

ISO 4720:2009 nomenclature: *Lavandula angustifolia* Mill. *Lavandula officinalis* Flower Oil is an essential oil obtained from the fresh flowering tops of the Lavender, *Lavandula officinalis* (syn: *L. vera*), *Labiatae*. It contains 30-40% esters calculated as linalyl acetate, linalool, pinene, limonene, geraniol, some eucalyptol (cineol) (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40370, last accessed 2009-11-09). According to Surburg/Panten, lavender oil is obtained by steam distillation of freshly cut flowering tops of Lavandula angustifolia Mill. (Lamiaceae). Main constituents according to GC are linalyl acetate (25-45%), cis-ocimene (4-10%), trans-ocimene (1.5-6%), 1,8-cineole (≤ 1%) camphor (≤ 0.5%), linalool (25-38%), 1-terpinen-4-ol (2-6%) and lavandulyl acetate [25905-14-0] (≥ 2%) (34).

In addition to distillation, both Lavandula officinalis and Lavandin are also solvent extracted, yielding concretes and, after ethanol extraction, absolutes, which are said to have a longer-lasting odour (34).

For Oil of lavender (Lavandula angustifolia Mill.) an ISO standard exists: ISO 3515:2002.

LAVANDULA SPICA *HERB OIL*

CAS 97722-12-8; EC 307-762-6 *(Lavender, Lavandula spica, ext. = INCI*

"Spike Oil"

Current regulation: …

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to "spike" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=8 (9.3%) positive reactions to "spike" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: Lavandula latifolia Medik. Lavandula Spica Herb Oil is an essential oil distilled from the flowering herbs of the Spikenard, *Lavandula spica* (syn: *Lavandula latifolia*), *Labiatae*. It contains eucalyptol (35%), camphor, linalool, borneol, terpineol, D-camphene and sesquiterpenes (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40372, last accessed 2010-01-29). According to Surburg/Panten, Spanish spike lavender oil is steam distilled from the flowering tops of Lavandula latifolia Medik.. The main components are linalool (34-50%), 1,8-cineole (16-39%) and camphor (8-16%) (34). For Oil of spike lavender (Lavandula latifolia (L.f.) Medikus), Spanish type an ISO standard exists: ISO 4719:1999

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

LITSEA CUBEBA *FRUIT EXTRACT*

CAS 90063-59-5, 68855-99-2; EC 290-018-7 *(Litsea cubeba, ext.) INCI: LITSEA CUBEBA OIL*

Current regulation: …

Clinical data:

The Rudzki 1976 study found 3 positive reaction in 200 patients to "Litsea cubeba" essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=7 (8.1%) positive reactions to this essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Litsea cubeba* (Lour) Pers. Litsea Cubeba Fruit Extract is an extract obtained from the fruits of the plant, *Litsea cubeba*, *Lauraceae* (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40036, last accessed 2009-11-24. The content by GC is: neral (25-33%), geranial (38-45%) – i.e. about ¾ citral, for which the extract had previously served as a raw material (34); direct use for perfuming is limited to household products (39). For Oil of Litsea cubeba (Litsea cubeba Pers.) an ISO standard exists: ISO 3214:2000.

In a LLNA study by RIFM, the "Litsea cubeba oil" as used was reported to contain 85.7% citral, 2.9% limonene, 1.7% linalool, 1.4% citronellal and < 1% caryophyllene and methyl heptanone, according to analyses of the supplier. The EC3 value was calculated as 8.4 % (227).

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

MENTHA ARVENSIS *LEAF OIL*

Cornmint oil

CAS 68917-18-0 ; EC /

INCI: MENTHA ARVENSIS OIL

Current regulation: /

Clinical data: /

Additional information:

Mentha Arvensis Leaf Oil is the oil derived from the leaves of the Horse Mint, Mentha arvensis L., Labiatae http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=57860

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

MENTHA PIPERITA *OIL*

CAS 8006-90-4; EC / (Oils, peppermint) INCI: MENTHA

PIPERITA OIL / 84082-70-2; EC 282-015-4 *(Peppermint, ext.) INCI names: MENTHA PIPERITA …*

Peppermint oil

Current regulation: /

Clinical data:

In the Frosch 2002 b study, 0.6% of 1606 consecutive patients reacted positively to “peppermint oil (American)”, tested 2% in pet. (17). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 2 positive reactions to "peppermint oil" were observed (3). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=1 (0.1%) positive reactions to peppermint oil 2% pet. (22). Among 512 patients referred from a dental department for diagnostic work-up of various intraoral symptoms and complaints within 4 years, 6 patients had positive (+ to +++) PT reactions to “peppermint oil” 1% pet. at D4, mostly combined with positive reactions to menthol (see above) and reporting dramatic improvement after cessation of use of peppermint-containing oral products (154). The Rudzki 1976 study found 1 positive reaction in 200 patients to “Peppermint” essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=6 (6.9%) positive reactions to “peppermint” essential oil 2% pet. (27). In 63 patients positive to the FM I, 3 had positive PT reactions to peppermint oil, 2% pet., in the Santucci 1987 study (28). The IVDK 2010 c study identified 0.6% positive reactions in 6546 patients tested in the context of a special series (30).

An unusual case of “baboon-like” allergic contact dermatitis of the vulva after drinking excessive amounts of a herbal tea containing, among other ingredients, peppermint. While the PT reaction to peppermint oil was only weak to doubtful, dramatic improvement after cessation and prompt relapse after repeat ingestion proved the diagnosis (243). Recurrent foot and lower leg dermatitis after the application of a “foot spray” (containing peppermint oil) was diagnosed as allergic contact dermatitis due to this ingredient in a 59 year old golf player (244). In another case, ACD after application of a transdermal system for the treatment of lumbar pain was attributed to CA to peppermint oil (2% pet.) and its main ingredient menthol (1% pet.) (155). In a patient with toothpaste-induced cheilitis, not only *M. piperita*, but also *M. arvensis*, but not *M. spicata* or *cardica* extracts (all tested 1% pet.), as well as natural and synthetic menthol caused positive PT reactions (245).

Additional information:

ISO 4720:2009 nomenclature: Mentha x piperita L. A standard by ISO exists for Oil of peppermint (Mentha x piperita L.): ISO 856:2006. A review by the Cosmetic Ingredient Review Expert Panel, Washington, DC on the “Final report on the safety assessment of Mentha Piperita (Peppermint) Oil, Mentha Piperita (Peppermint) Leaf Extract, Mentha Piperita (Peppermint) Leaf, and Mentha Piperita (Peppermint) Leaf Water“ is available (163), stating that “Peppermint Oil is used at a concentration of < or = 3% in rinse-off formulations and < or = 0.2% in leave-on formulations. Peppermint Oil is composed primarily of menthol and menthone. Other possible constituents include pulegone, menthofuran, and limone. According to Surburg/Panten: (-)-menthol (34-46%), (-)-menthone (15-27%), (-)-menthyl acetate (2.5-7%) and menthofuran [17957-94-7] (0.5-6%) (34). According to (30) the maximum observed concentration in peppermint oil are (in %): (-)-menthol (49); (-)-menthone (28); (-)-menthyl acetate (8); mentofuran (8); isomenthone (8); neo menthol (6); pulegone (3.5); limonene (3.0); linalool (0.4) (30). Most of the safety test data concern Peppermint Oil. The oil is considered to present the "worst case scenario" because of its many constituents, so data on the oil were considered relevant to the entire group of ingredients. … Repeated

intradermal dosing with Peppermint Oil produced moderate and severe reactions in rabbits ….” concluding that “with the limitation that the concentration of pulegone in these ingredients should not exceed 1%, it was concluded that Mentha Piperita (Peppermint) Oil, Mentha Piperita (Peppermint) Extract, Mentha Piperita (Peppermint) Leaves, Mentha Piperita (Peppermint) Water are safe as used in cosmetic formulations”.

MENTHA SPICATA *HERB OIL*	*CAS* 84696-51-5, 8008-79-5; EC 283-656-2 (Spearmint, ext.)
Spearmint oil	*INCI: MENTHA VIRIDIS EXTRACT*

Current regulation: /

Clinical data:

In the Frosch 2002 b study, 0.8% of 1606 consecutive patients reacted positively to “spearmint oil (American)”, tested 2% in pet. (17). The CAS # quoted (8008-79-5) refers, according to CosIng, to MENTHA VIRIDIS LEAF OIL, the volatile oil obtained from the dried tops and leaves of the Garden Mint, Mentha viridis L., Labiatae. The Larsen 2001 study diagnosed 5.0% positive reactions in 178 patients with known contact allergy to fragrance ingredients, using this oil at 5% pet. test concentration (19). In the An 2005 study, 6 of 422 consecutive patients, i.e., 1.4%, had positive reactions to “Mentha viridis oil” 5% (13). PT results with toothpaste ingredients were positive in 7 patients, of whom 4 had strong positive reactions to spearmint (246).

Additional information:

ISO 4720:2009 nomenclature: Mentha spicata L. Mentha Spicata Oil is an essential oil obtained from the herbs of the Spearmint, Mentha spicata L., Labiatae (syn: Mentha viridis L., Labiatae). It contains carvone (more than 50%), limonene, pinene (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40394, last accessed 2009-11-11). According to Surburg/Panten, the content is limonene (9-16.5%), (-)-carvone (60-70%), menthone (0-0.2%) and viridiflorol (0-0.5%) (34). Exposure by toothpastes, and subsequent contact allergic reaction of the lips or the oral mucosa, have been reported (e.g., (247, 248)). L-Carvone is a component of the oil from Mentha spicata (spearmint) (53) and had been tested with positive results in “toothpaste cases”, even at a concentration as low as 0.067% (68).

For Oil of spearmint -- Part 1: Native type (Mentha spicata L.) an ISO standard exists: ISO 3033-1:2005, for Oil of spearmint -- Part 2: Chinese type (80 % and 60 %) (Mentha viridis L. var. crispa Benth.), redistilled oil: ISO 3033-2:2005, for Oil of spearmint -- Part 3: Indian type (Mentha spicata L.), redistilled oil: ISO 3033-3:2005 and for Oil of spearmint -- Part 4: Scotch variety (Mentha x gracilis Sole): ISO 3033-4:2005.

MYROXYLON PEREIRAE *RESIN*	CAS 8007-00-9; EC 232-352-8 (Balsams, Peru)
Balsam of Peru	INCI: MYROXYLON PEREIRAE / Balsams, Peru

Current regulation: Annex III, part1, n° 154

Clinical data:

This natural mixture has been employed as screening agent in Baseline series worldwide for many decades. Hence, a wealth of data is available; table 3.2 – 1 summarises results of the past 10 years.

Additional information:

ISO 4720:2009 nomenclature: *Myroxylon pereirae (Royle) Klotzsch, syn. Myroxylon balsamum var. pereirae (Royle) Harms. Myroxylon pereirae resin* (MPR, Balsamum peruvianium) is harvested from the balsam of Peru tree, *Myroxylon balsamicum* (L.) HARMS *var. pereirae* (ROYLE) HARMS, synonymous *Myroxylon pereirae* (ROYLE) KLOTZSCH (249) after thermal stress, almost exclusively in El Salvador. Main constituents of the pleasantly, vanilla-like smelling dark brown liquid are benzyl esters of cinnamic and benzoic acid (35 – 75%), up to 30% cinnamic acid, up to about 10% benzoic acid, approximately 5% alpha- and beta-nerolidol, benzyl alcohol and mostly less than 1% cinnamyl alcohol, benzyl ferulate and -isoferulate, cinnamic acid amyl ester, coniferyl alcohol, coniferyl benzoate, eugenol, isoeugenol, farnesol, vanillin, and several trace constituents (250-253). The composition of MPR varies with the origin and other factors; moreover, MPR is sometimes blended with other natural mixtures such as turpentine, styrax or colophonium (249).

MPR can be used to improve taste or smell in gargling solutions, cosmetic products such as soaps, shampoo or lipsticks, as well as sweets, tobacco and beverages (249, 254). According to EU legislation and IFRA guidelines MPR should not be used in products intended for skin contact; however, extracts and distillates of MPR may be used in a concentration of < 0,4% (IFRA-Guidelines, www.ifraorg.org (255)). E. Temesvári et al. report on the interesting case of severe ACD with subsequent hypopigmentation after a "temporary henna tattoo", which was, unexpectedly, not due to p-phenylene diamine, but to the oil used to disperse the pigment, which presumably contained allergens also included in the FM I and MPR, both of which were extreme positive on a later PT (256).

In addition to delayed type hypersensitivity reactions, MPR (and some of his constituents such as benzoic acid (257)) are capable of eliciting (non-immunological) urticarial immediate reactions (258-260). In one case, the immediate reaction to MPR (and to FM I) at the test site spread systemically in terms of a generalised urticaria, while no delayed type reactions were observed to the PT (261). Generally, there is apparently no association of immediate reactions to MPR (and cinnamal or cinnamyl alcohol) and contact sensitisation to these compounds (262). In animal experiments the sensitising potency of MPR was clearly established (250), with coniferyl benzoate identified as single compound with the most marked potency (252). However, due to the limited chemical stability of this compound is is unclear whether other, more stable compounds are, in fact, more important allergens, such as cinnamic acid and (iso-) ferulic acid esters or oxidised constituents of the resin fraction (263).

Table 3.2.2 – 1: Results with contact allergy to fragrance ingredients screening agents reported since 1999 in patients patch tested for suspected allergic contact dermatitis: **Myroxylon pereirae resin** (Balsam of Peru) 1). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the reviewers.

Country	Population	Years	No. tested	Crude % positive (95% CI) [§]
Tel Aviv, Israel (264) #	Consecutive patients	1999-2000	943	6.6 % (5.1 – 8.4) [§]
South Korea (13)	Consecutive patients	04/2002 – 06/2003	422	7.3% (5.1 – 10:3%) [§]

Tel Aviv, Israel (265)	Consecutive patients	1998-2004	2156	3.6 % (2.9 – 4.5) §
Manipal, India (266)	Dermatitis patients	1989-1998	1780	n=17
Tehran, Iran (267)	Consecutive patients	2002-2004	250	2.4 % (0.9 – 5.2) §
Sevilla, Spain (268)	Consecutive patients	2002-2004	863	5.8 % (4.3 – 7.6) §
Ankara, Turkey (269)	Consecutive patients	1992-2004	1038	2.1 % (1.3 – 3.2) §
Vienna, Austria (22)	Consecutive patients of one clinic	1997-2000	2660	5.4% (4.6 – 6.3%) §
Czech Republic (270)	Consecutive patients	1997-2001	12058	7.3% (6.8 – 7.8) §
Copenhagen, Denmark (271)	Consecutive patients	1985-2007	16173	3.9 % (3.6 – 4.2) §
Sweden (272)	Consecutive patients	2000	3790	6.5%
9 European countries (273) $	Consecutive patients	2002-2003	9672	6.1 %
Germany, 3 Swiss + 1 Austrian Dept. (7)	Consecutive patients	2005-2008	36919	8.0% (7.7 – 8.3%)
10 depts. From 7 EU countries (274) *	Consecutive patients	1996-2000	26210	6.0 %
USA (Canada) (20)	Probably consecutive patients	2003	1603	6.6%
NACDG 2009 (21)	Consecutive patients	2005-2006	4449	11.9%

§ Calculated by reviewers, where possible (if actual numbers were given)

Probably included in (265)

$ > 5-fold difference between departments

* About 4-fold difference between departments

NARCISSUS SPP. *EXTRACT / OIL* *CAS: diverse*

Narcissus abs.

Current regulation: /

Clinical data:

In the Frosch 2002 b study, 1.3% positive reactions to "narcissus absolute" (2% pet.) were observed in 1606 consecutive (17). The extract used by the PT allergen provider Almirall/Hermal/Trolab has the CAS number 90064-25-8. The IVDK 2010 c study identified 0.5% positive reactions in 2445 consecutively tested patients and 0.6% positive reactions in 809 patients tested in the context of a special series (30).

Additional information:

Commonly used: *Narcissus poeticus* L. According to (30) the maximum observed concentration in Narcissus abs. are (in %): α-terpineol (23.7); trans-Isoeugenol methyl ether (20); benzyl benzoate (20); coumarin (5.7); benzyl alcohol (4.0); Δ^3-carene (3.4); cinnamyl alcohol (2.5); phenylethyl alcohol (2.2); ethyl palmitate (2.2); phenylpropyl acetate (1.7); 1,8-cineole (1.5); caryophyllene (1.0); benzyl acetate (0.7); isoeugenol (0.5); farnesol (0.3) (also according to (17)) (30).

OCIMUM BASILICUM *HERB OIL*

CAS 84775-71-3; EC 283-900-8 *(Ocimum basilicum, ext. = INCI)*

Basil Oil (sweet)

Current regulation: /

Clinical data:

/

Additional information:

ISO 4720:2009 nomenclature: *Ocimum basilicum* L. For Oil of basil, methyl chavicol type (Ocimum basilicum L.) an ISO standard exists: ISO 11043:1998. *Ocimum Basilicum* Herb Oil is an essential oil obtained from the herbs of the Sweet Basil, *Ocimum basilicum* L., *Labiatae*. (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40474, last accessed 2009-11-24). The chemical composition varies greatly with the origin (34):

- Basil oil of the methylchavicol type (Réunion type) is extracted from flowering tops or whole plants from Réunion, Comores, Madagascar, but also other countries such as Egypt. Mainly used for seasoning food. Content by GC: methylchavicol 75-87%, linalool 0.5-3%
- Basil oil, linalool type is produced mainly in the Mediterranean aera. Content by GC: Linalool 45-62%, methylchavicol trace to 30%, eugenol 2-15%
- Indian Basil oil is produced exclusively in India. Content by GC: methylchavicol trace to 70%, linalool 25%.

In a LLNA study by RIFM, the basil oil as used was reported to contain 51% linalool, 10.4% eugenol, 7.7% cineol, 3.7% bergamotene, 2.7% germacrene D, 2.7% cadinol and 1.3% cadinene, according to analyses of the supplier. The EC3 value was calculated to be < 2.5% (227).

PELARGONIUM GRAVEOLENS *FLOWER OIL*

CAS 90082-51-2; EC 290-140-0 *(Pelargonium graveolens, ext. = INCI) / 8000-46-2; EC / (Oils, geranium) INCI: GERANIUM*

Geranium Oil Bourbon

Current regulation: /

Clinical data:

The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "geranium oil Bourbon" (2% pet.) 7.4% positive reactions (9). In the Larsen 2001 study, 8.4% positive reactions were observed in 178 patients with known contact allergy to fragrance ingredients ("geranium oil Bourbon", 10% pet.) (19). The Goossens 1997 study found 3 of 111 patients positive to “geranium oil 20% pet.” – all sensitised to other fragrance allergens (23). The Rudzki 1976 study found 3 positive reactions in 200 patients to “geranium” essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=2 (2.3%) positive reactions to “geranium” essential oil 2% pet. (27). Nakayama et al. found 1974 (after (29)) 3 “strong positive” reactions to “Geranium oil” (unknown test concentration) in 183 patients, Trattner/David 1 / 641 consecutive patients positive to “Geranium oil” (31). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=8 reacted positively to geranium oil bourbon (48).

Additional information:

ISO 4720:2009 nomenclature: *Pelargonium x ssp.* For Oil of geranium (Pelargonium X ssp.) an ISO standard exists: ISO 4731:2006 Pelargonium Graveolens Flower Oil is the volatile oil obtained from the flowers of the Bourbon Geranium, *Pelargonium graveolens* L. Hér. Ex Aiton, *P. roseum* Willdenow (and other nondefined hybrids that have developed in different regions of the world) Geraniaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details &id=57527, last accessed 2009-11-16)(34). The Bourbon type (Réunion, Madagascar) is more valuable than the North African and Chinese products, and differs in characteristic components: (-)-6,9-guaiadiene [36577-33-0] 5-9% in the Bourbon type, and 10-epi-gamma-eudesmol [15051-81-7] 3-6% in the African type, in addition to the main components (-)-citronellol, isomenthone, formates and tiglates. Chinese oil is similar to Bourbon oil, however, it contains more citronellol (32-43%) and lower amounts of linalool (2-4.5%) and geraniol (5-12%) (34).

In a LLNA study by RIFM, the geranium oil as used was reported to contain 41.1% citronellol, 9.8% 2,6-guiadine, 6.2% isomethone, 4.9% geraniol, 2.2% cis-rose oxide, 2.1% linalool, 1.5% geranyl formate, 1.3% phenyl ethyl tiglate, 1.0% trans-rose oxide, and geranyl tiglate and alpha-pinene at < 1%, according to analyses of the supplier. The EC3 value was calculated to be > 50% (227).

PELARGONIUM ROSEUM *LEAF OIL*

CAS 90082-55-6; EC 290-144-2 *(Pelargonium roseum, ext. = INCI)*

Geranium Oil; Rose Geranium Oil

Current regulation: /

Clinical data:

In the Sugiura 2000 study, among 1483 patients with suspected cosmetic dermatitis, 2.1% positive PT reactions to "geranium oil" (tested 20% in pet.) were observed (14).

Additional information:

Pelargonium Roseum Leaf Oil is an essential oil obtained from the leaves of the plant, Pelargonium roseum, Geraniaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40565, last accessed 2009-11-16.

PIMENTA RACEMOSA LEAF/FRUIT OIL CAS 85085-61-6; EC 285-385-5

Bay oil (34)

Current regulation: /

Clinical data: /

Additional information:

ISO 4720:2009 nomenclature: *Pimenta racemosa* (Mill.) J.W. Moore. For Oil of bay [Pimenta racemosa (Mill.) J.W. Moore] an ISO standard exists: ISO 3045:2004 Pimenta Racemosa Leaf/Fruit Oil is an essential oil obtained from the fruits of the plant, Pimenta racemosa, Myrtaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41014, last accessed 2010-02-10).

Steam distillation of the leaves of Pimenta racemosa (Mill.) J.W. Moore (Myrtaceae) yields bay oil, which consists of myrcene (20-30%), eugenol (42-56%) and chavicol (8-13%) (34).

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

Pinus mugo *leaf and twig oil and extract* *CAS 90082-72-7, 8000-26-8; EC* 290-163-6

Dwarf pine needle oil (German: Latschenkiefernöl)

Current regulation: Annex III, part 1, 109

Clinical data:

In the Frosch 2002 b study, 0.7% positive reactions to dwarf pine needle oil (2% pet.) were observed in 1606 consecutive (17). The Rudzki 1976 study found 4 positive reactions in 200 patients to “Pine needle” essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to “pine needle” essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Pinus mugo* Turra syn. *Pinus montana* Mill.) Pinus Mugo Twig Oil is an essential obtained from the twigs of the Pine, Pinus mugo, Pinaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41476&back=1, last accessed 2010-03-09). Pinus Mugo Twig Leaf Extract is an extract obtained from the twigs leaves of the Pine, Pinus mugo, Pinaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=searc

h.details&id=41473&back=1, last accessed 2010-03-09).

Dwarf pine needle oil is obtained from *Pinus mugo* Turra subsp. *mugo* and subsp. *pumilio* (Haenke) Franco (34). For Oil of dwarf pine (Pinus mugo Turra) an ISO standard exists: ISO 21093:2003. American pine oils contain almost no 3-carene or camphene (34).

PINUS PUMILA *TWIG LEAF EXTRACT / OIL*

CAS 97676-05-6; EC 307-681-6 *(Pine, Pinus pumila, ext. = INCI)*

Dwarf pine needle oil

Current regulation: Annex III, part 1, 114

Clinical data: /

Additional information:

Pinus Pumila Twig Leaf Extract obtained from the twigs leaves of the Pine, Pinus pumila, Pinaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41483&back=1, last accessed 2009-11-12), Pinus Pumila Twig Leaf Oil is the essential oil obtained from the twigs leaves of the Pine, Pinus pumila, Pinaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41484&back=1, last accessed 2009-11-12). Main constituents are alpha-pinene (60-70%) and beta-pinene (20-25%). (34) Occurrence from Siberia to Japan, classified as Endangered Species

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

POGOSTEMON CABLIN *OIL*

CAS 8014-09-3; EC / (Oils, patchouli) / 84238-39-1; EC 282-493-4 *(Patchouli, ext.)*

Patchouli oil

INCI: POGOSTEMON CABLIN / *Patchouli, ext.*

Current regulation: /

Clinical data:

In the Frosch 2002 b study, 0.8% positive reactions to patchouli oil (10% pet.) in 1606 consecutive were observed (17). Nakayama et al. found 1974 (after (29)) 3 “strong positive” and 8 “weak positive” reactions to “Patchouli oil” (unknown test concentration) in 183 patients. The IVDK 2010 c study identified 0.6% positive reactions in 2446 consecutively tested patients and 1.4% positive reactions in 828 patients tested in the context of a special series (30).

Additional information:

ISO 4720:2009 nomenclature: *Pogostemon cablin* (Blanco) Benth. syn. *Mentha cablin* Blanco. An ISO standard is available for Oil of patchouli (Pogostemon cablin (Blanco) Benth.): ISO 3757:2002. Pogostemon Cablin Leaf Oil is an essential oil obtained from the fermented leaves of the Patchouli, *Pogostemon cablin* (syn: *Pogostemon patchouli*),

Labiatae (Lamiaceae (34)). It contains patchouli alcohol, beta-patchoulene, azulene, eugenol, sesquiterpenes (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40927, last accessed 2009-11-12). Although the sesquiterpene alcohol (-)-patchoulol [5986-55-0] is the main component of patchouli oil (27-35%), the compound largely contributing to the characteristic odour is norpatchoulenol [41429-52-1] (0.35-1%). Other constituents include (+)-alpha-bulnesene [6391-11-0] (13-21%), (-)-alpha-guajene [3691-12-1] (11-16%), (-)-ß-patchoulene [514-51-2] (1.8-3.5%) and (-)-seychellene [20085-93-2] (1-3%) (34). According to (30) the maximum observed concentration in patchouli oil are (in %): (-)-patchoulol (35); (+)-alpha-lulnesene (21); (-)-alpha-guajene (16); β-pinene (6); (-)-ß-patchoulene (3.5); (-)-seychellene (3); pogostol (2.5); α-pinene (2.5); norpatchoulenol (1) (30).

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

ROSE FLOWER OIL (ROSA SPP.)	*CAS 8007-01-0; EC / (Oils, rose)*
ROSA ALBA FLOWER EXTRACT	*CAS 93334-48-6; EC* 297-122-1 *(Rose, Rosa alba, ext. = INCI)*
ROSA CANINA FLOWER OIL	*CAS 84696-47-9; EC* 283-652-0 *(Rose, Rosa canina, ext.) INCI: ROSA CANINA*
ROSA CENTIFOLIA FLOWER OIL	*CAS 84604-12-6, EC* 283-289-8 *(Rose, Rosa centifolia, ext.) INCI: ROSA CENTIFOLIA / Rose, Rosa centifolia, ext.*
ROSA DAMASCENA FLOWER OIL	*CAS 90106-38-0; EC* 290-260-3 *(Rose, Rosa Damascena, ext. = INCI)*
ROSA GALLICA FLOWER OIL	*CAS 84604-13-7; EC* 283-290-3 *(Rose, Rosa Gallica, ext.) INCI: ROSA GALLICA*
ROSA MOSCHATA OIL	*--*
ROSA RUGOSA FLOWER OIL	*CAS 92347-25-6; EC* 296-213-3 *(Rose, Rosa rugosa, ext.)*

Current regulation: /

Clinical data:

In the Sugiura 2000 study, 1483 patients with suspected cosmetic dermatitis were PTed with "rose oil Bulgaria" (2% pet.), yielding 0.4% positive reactions (14); Trattner/David found 2 / 641 consecutive patients positive to “Rose oil (Bulgarian)” (31). The Bulgarian rose oil usually corresponds to Rosa Damascena Flower Oil (http://en.wikipedia.org/wiki/Rose_oil, last accessed 2009-11-16). The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "rose Bulgarian oil" (2% pet.) 4.5% positive reactions (9). One case of contact allergy to “Bulgarian rose oil (2 % pet.)” – and geraniol – in a 48-year-old female with ACD after application of “Eau de Rochas” E.d.C. was diagnosed, among 326 patients with suspected contact allergy to fragrance ingredients had tested negative (275). However, other rose oils are also used (and capable of eliciting ACD) as illustrated by the case of a 27 year old woman who developed ACD after using “Rose Absolute Eau ® eau de

parfum", a "non-scented" body lotion and a number of other topicals. PTing revealed a number of (previously) relevant reaction, including "Rose centifolia" (5% alc.) and "Rose oil Bulgarian" (2% pet.) essential oil preparations (276). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had positive reactions to "Rose oil Bulgarian", tested at 2% concentration (13). Nakayama et al. found 1974 (after (29)) 4 "strong positive" reactions to "Rose oil Bulgarian" (unknown test concentration) in 183 patients. In a study from Alicante, Spain, 86 selected patients were tested with rose oil absolute, yielding 6 positive reactions (48).

Additional information:

ISO 4720:2009 nomenclature: Rosa x damascena Mill. and Rosa sertata X Rosa rugosa. Rose Flower Oil is the volatile oil obtained from the flowers of Rosa spp. , rosaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details &id=59362, last accessed 2009-11-16). "Rose oil, meaning either rose otto (attar of rose, attar of roses) or rose absolute, is the essential oil extracted from the petals of various types of rose. Rose ottos are extracted through steam distillation, while rose absolutes are obtained through solvent extraction or supercritical carbon dioxide extraction, with the absolute being used more commonly in perfumery" (http://en.wikipedia.org/wiki/Rose_oil, last accessed 2009-11-17) There are several more specifically named flower extracts used for masking or perfuming:

- Rosa Alba Flower Extract is an extract obtained from the flowers of the Rose, Rosa alba L., Rosaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40969, last accessed 2009-11-16).
- Rosa Canina Flower Oil is the volatile oil obtained from the flowers of the Hip Rose, Rosa canina L., Rosaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=59263, last accessed 2009-11-16).
- Rosa Centifolia Flower Oil is the volatile oil obtained from the flowers of the Cabbage Rose, Rosa centifolia (L.), Rosaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=79757, last accessed 2009-11-16).
- Rosa Damascena Flower Oil is the volatile oil obtained from the flowers of the Damask Rose, Rosa damascena, Rosaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=79760, last accessed 2009-11-16).
- Rosa Gallica Flower Oil is the volatile oil obtained from the flowers of the French Rose, Rosa gallica L., Rosaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=59346, last accessed 2009-11-16).
- Rosa Moschata Oil is the oil obtained from the Musk Rose, Rosa moschata, Rosaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=79761, last accessed 2009-11-16).
- Rosa Rugosa Flower Oil is the volatile oil obtained from the flowers of the Rose, Rosa rubiginosa L., Rosaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=83588, last accessed 2009-11-16).

Apparently, the Rosa Damascena and the Rosa centifolia are the species most commonly used for extraction of essential rose oils, the former mostly grown in Bulgaria, Turkey, Russia, India and China, the latter more commonly in Morocco, France and Egypt (276). Main constituents by GC are: citronellol (20-49%), geraniol (6-23%), nerol (3-12%) and phenylethyl alcohol (up to 3.5%) (34).

For Oil of rose (Rosa x damascena Miller) an ISO standard exists: ISO 9842:2003.

ROSMARINUS OFFICINALIS *FLOWER OIL*	*CAS 84604-14-8; EC* 283-291-9 *(Rosemary, ext.)*
"Rosemary Oil"	*INCI: ROSMARINUM OFFICINALIS / Rosemary, ext.*

Current regulation: /

Clinical data:

The Rudzki 1976 study found no positive reaction in 200 patients to “rosemary” essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to “rosemary” essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Rosmarinus officinalis* L. Rosmarinus Officinalis Flower Oil is an essential oil obtained from the leaves and fresh flowering tops of the Rosemary, *Rosmarinus officinalis* L., Lamiaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40978, last accessed 2010-01-29). Major constituents are: 1,8-cineole (17-55%), alpha-pinene (9-26%), camphor (5-22%) and verbenone [18309-32-5] as traces in North African oils, but between 0.7 and 2.5% in Spanish oils (34). For Oil of rosemary (Rosmarinus officinalis L.) an ISO standard exists: ISO 1342:2000.

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

SALVIA spp. *HERB OIL*

Sage oil

SALVIA OFFICINALIS LAVANDULIFOLIA *HERB OIL*	*CAS 97952-71-1; EC* 308-365-0 *(Sage, Salvia officinalis lavandulifolia, ext. = INCI)*
SALVIA LAVANDULIFOLIA *HERB OIL*	*CAS 90106-49-3; EC* 290-272-9 *(Sage, Salvia lavandulifolia, ext. = INCI)*
SALVIA SCLAREA *FLOWER OIL*	*CAS 84775-83-7; EC* 283-911-8 *(Sage, Salvia sclarea, ext.) INCI: SALVIA SCLAREA / Sage, Salvia sclarea, ext.*
SALVIA HISPANICA *HERB OIL*	*CAS 93384-40-8; EC* 297-250-8 *(Sage, Salvia hispanica, ext. = INCI)*

Current regulation: /

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to “Clary sage”, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=4 (4.6%) positive reactions to “clary sage” essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Salvia officinalis* L. *Salvia Officinalis Lavandulifolia* Herb Oil is an essential oil obtained from the herbs of the Sage, *Salvia officinalis* L. *spp. lavandulifolia*, *Lamiaceae*, Syn. Dalmatian sage (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41084, last accessed 2010-01-29).

Salvia Lavandulifolia Herb Oil is an essential oil obtained from the herbs of the Sage, *Salvia lavandulifolia*, *Lamiaceae* (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40987, last accessed 2010-01-29).

Salvia Sclarea Flower Oil is an essential oil obtained from the flowers and foliage of the Clary Sage, *Salvia sclarea* L., *Lamiaceae* (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41086, last accessed 2010-01-29).

Salvia Hispanica Herb Oil is an essential oil obtained from the herbs of the Spanish Sage, *Salvia hispanica* L., *Lamiaceae* (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40985, last accessed 2010-01-29).

Clary sage oil is obtained by steam distillation of flowering tops and foliage of cultivated *Salvia sclarea* L. (Lamiaceae). Main constituents are linalyl acetate (56-78%) and linalool (6.5-24%) (34). Dalmatian sage oil is steam distilled from partially dried leaves of S. officinalis L. (Lamiaceae). The content by GC is: alpha-thujone (18-43%), beta-thujone (3-8.5%), 1,8-cineole (5.5-13%), camphor (3-8.5%) as main constituents (34). Spanish sage oil does not contain thujone, but mainly camphor (15-36%) and 1,8-cineole (11-30%), and is used mainly in pharmaceutical preparations and technical perfumery (34). For Oil of sage, Spanish (*Salvia lavandulifolia* Vahl) an ISO standard exists: ISO 3526:2005, for Oil of Dalmatian sage (Salvia officinalis L.): ISO 9909:1997.

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

SANTALUM ALBUM *WOOD OIL*

CAS 84787-70-2; EC 284-111-1 *(Sandalwood, ext.) INCI: SANTALUM ALBUM / Sandalwood, ext.*

Sandalwood oil ([East] India)

SANTALUM ALBUM *OIL*

CAS 8006-87-9; EC / (Oils, sandalwood)

Sandalwood oil ([East] India)

Current regulation: /

Clinical data:

In the Sugiura 2000 study, 1483 patients with suspected cosmetic dermatitis were PTed with "sandalwood oil" (2% pet.), yielding 0.8% positive reactions (14). In the Frosch 2002 b study, “sandalwood oil (East India)” is mentioned with a CAS # 8015-65-4, which, however, is attributed to AMYRIS BALSAMIFERA BARK OIL, see above. Assuming that this CAS # is erroneous, study results are considered to be valid for S. album wood oil, tested at 2% and 10% concentration, yielding 0.4% and 0.9% positive reactions,

respectively (17). Out of 6 of 15 patients with a positive reaction to the higher concentration no clinical relevance was found, compared to 2 of 7 patients positive to the lower concentration (17). The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "sandalwood oil" (2% pet.) 6.6% positive reactions (9). In the An 2005 study, 10 of 422 consecutive patients, i.e., 2.4%, had positive reactions to “Santalum album oil” 2% (13). The Goossens 1997 study found 4 of 111 patients positive to “sandalwood oil 10% pet.” – all sensitised to other fragrance allergens (23). The Rudzki 1976 study found no positive reaction in 200 patients to “sandalwood”, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=2 (2.3%) positive reactions to “sandalwood” essential oil 2% pet. (27). In 63 patients positive to the FM I, 1 had a positive PT reaction to sandalwood oil, 2% pet., in the Santucci 1987 study (28). Nakayama et al. found 1974 (after (29)) 6 “strong positive” and 8 “weak positive” reactions to “Sandalwood oil” (unknown test concentration) in 183 patients. The IVDK 2010 c study identified 1.3% positive reactions in 3671 consecutively tested patients and 1.8% positive reactions in 1002 patients tested in the context of a special series (30). In a study from Alicante, Spain, 86 selected patients were tested with sandalwood oil, yielding 2 positive reactions (48).

Additional information:

ISO 4720:2009 nomenclature: *Santalum album* L. *Santalum Album* Oil is the volatile oil obtained from the heartwood of the Sandalwood, *Santalum album* L., Santalaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=80209, last accessed 2009-11-26).

Santalum Album Wood Oil is an essential oil obtained from the wood of the Sandalwood, *Santalum album* L., *Santalaceae*. It contains 75% santalol isomers (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41092, last accessed 2009-11-12), typically up to 55% .alpha.-santalol and up to 24% .beta.-santalol (30). East Indian sandalwood oil consists almost exclusively of closely related sesquiterpenoids; by far the main constituents are the alcohols alpha-santalol [115-71-9] (41-55%) and cis-beta-santalol [77-42-9] (16-24%), the latter being mainly responsible for the specific odour (34, 39).

An ISO standard regarding the composition of “*Santalum album* oil” is available: ISO 3518:2002. “Sandalwoods” are labelled as Amyris balsamifera, Eremophila mitchelli, Fusanus acuminatus (= Santalum acuminatum), Santalum album, S. austrocaledonicum, S. latifolium, S. spicatum and S. yasi. The majority of currently available trade oils, reportedly from S. album, contained approximately 50-70% santalols (Z-alpha and Z-beta), as analysed with gas chromatography-mass spectrometry (GC-MS) (277). A review on the toxicological properties of “Santalum album oil” is available (278).

AMYRIS BALSAMIFERA *BARK OIL (Sandalwood oil (Carribean)), CAS 8015-65-4; EC / (Oils, amyris) / 90320-49-3; EC* 90320-49-3 *(Amyris balsamifera, ext. = INCI name)* is used as a cheap substitute for East Indian Sandalwood in perfumes and cosmetics. Originally cultivated primarily in Haiti where it was known as 'candle wood' and used as a torch by locals due to the tree's high oil content (http://www.amphora-retail.com/sandalwood-amyris-essential-10ml-p-107.html, last accessed 2009-11-12). The major components are sesquiterpenoids such as valerianol, elemol, ß-eudesmol and epi-gamma-eudesmol (39). For Oil of amyris (Amyris balsamifera L.) an ISO standard exists: ISO 3525:2008. Amyris Balsamifera Bark Oil is the volatile oil distilled from the bark of the tree, Amyris balsamifera, Rutaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=74455, last accessed 2009-11-12).

SANTALUM SPICATA *WOOD OIL* *CAS 8024-35-9; EC* 296-618-5 *(Sandalwood oil, Western Australia)*

Sandalwood oil (Australia)

Current regulation: /

Clinical data:

In clinical studies, mostly S. album wood oil had been used (see above); in a number of studies this is not clear.

Additional information:

ISO 4720:2009 nomenclature: *Santalum spicatum* (R.Br.) A. DC, syn. *Eucarya spicata* (R.Br.) Sprag & Summ. For Oil of Australian sandalwood (*Santalum spicatum* (R.Br.) A.DC.) an ISO standard exists: ISO 22769:2009. Santalum Spicata Wood Oil is an essential oil obtained from the wood of the Australian Sandalwood, Santalum spicata, Santalaceae. It contains 75% santalols and 10% farnesol (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41093, last accessed 2009-11-12). This oil also contains santalols as main constituents but differs somewhat in the remaining composition. Today, it makes up a considerable part of the sandalwood oil market (34).

Considering the content of well-known allergenic compounds (santalols), this essential oil is regarded as established contact allergen in humans.

TAGETES PATULA *FLOWER OIL* *CAS 91722-29-1; EC* 294-431-3 *(Tagetes patula, ext. = INCI)*

"Marigold Oil; Tagetes Oil"

Current regulation: /

Clinical data:

In an aromatherapist, an essential oil solvent-extracted from *Tagetes patula*, patch tested at 1.5% in grapeseed oil (vehicle negative, 7 controls negative to essential oils) resulted in a +++ reaction, in accordance with a work-related bilateral hand dermatitis (217).

Additional information:

Tagetes Patula Flower Oil is an essential oil obtained by hydrodistillation of the flowers of the *Tagetes*, *Tagetes patula L., Compositae*. It contains mainly D-limonene, ocimene, 2,6-dimethyloct-7-en-4-one (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41506, last accessed 2010-01-28). According to Surburg/Panten, tagetes oil is steam distilled from the flowering plants of Tagetes minuta L. (T. glandulifera Schrank., Asteraceae). Main components comprise cis-ocimene, dihydrotagetone, tagetone, and cis- and trans-ocimenone (34, 39).

THYMUS spp. *HERB OIL*

THYMUS VULGARIS *HERB OIL*

CAS 84929-51-1, 8007-46-3; EC 284-535-7 *(Thyme, Thymus vulgaris, ext.)*

"Thyme oil"

INCI: THYMUS VULGARIS / Thyme, Thymus vulgaris, ext.

Current regulation: /

Clinical data:

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

Additional information:

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

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

TURPENTINE *(oil)*

CAS 8006-64-2 / 9005-90-7 / 8052-14-0; EC 232-350-7 / 232-688-5 / -

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

Clinical data:

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

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

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

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

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

Additional information:

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

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

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

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

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

Clinical data: /

Additional information:

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

VETIVERIA ZIZANOIDES *ROOT OIL*

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

"Vetiver oil; khas khas oil"

Current regulation: …

Clinical data:

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

Additional information:

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

Acknowledgement: We thank Erich Schmidt for critically reviewing the nomenclature of natural extracts and for providing ISO terminology.

References

1. Larsen W, Nakayama H, Fischer T, Elsner P, Frosch P, Burrows D, Jordan W, Shaw S, Wilkinson J, Marks J, Sugawara M, Nethercott M, Nethercott J. Fragrance contact dermatitis - a worldwide multicenter investigation (Part III). *Contact Dermatitis* 2002: 46: 141-144.

2. Hendriks S A, van Ginkel C J. Evaluation of the fragrance mix in the European standard series. *Contact Dermatitis* 1999: 41: 161-162.

3. Katsarma G, Gawkrodger D J. Suspected fragrance allergy requires extended patch testing to individual fragrance allergens. *Contact Dermatitis* 1999: 41: 193-197.

4. Schnuch A, Uter W, Geier J, Lessmann H, Frosch P J. Sensitization to 26 fragrances to be labelled according to current European regulation. Results of the IVDK and review of the literature. *Contact Dermatitis* 2007: 57: 1-10.

5. Temesvari E, Nemeth I, Balo-Banga M J, Husz S, Kohanka V, Somos Z, Judak R, Remenyik E V, Szegedi A, Nebenfuhrer L, Meszaros C, Horvath A. Multicentre study of fragrance allergy in Hungary. Immediate and late type reactions. *Contact Dermatitis* 2002: 46: 325-330.

6. van Oosten E J, Schuttelaar M L, Coenraads P J. Clinical relevance of positive patch test reactions to the 26 EU-labelled fragrances. *Contact Dermatitis* 2009: 61: 217-223.

7. Uter W, Geier J, Frosch P J, Schnuch A. Contact allergy to fragrances: current patch test results (2005 to 2008) from the IVDK network. *Contact Dermatitis* 2010: 63: 254-261.

8. Schnuch A, Geier J, Uter W, Frosch P J. Another look on allergies to fragrances: frequencies of sensitisation to the fragrance mix and its constituents. Results from the IVDK. *Exog Dermatol* 2002: 1: 231-237.

9. Brites M M, Goncalo M, Figueiredo A. Contact allergy to fragrance mix--a 10-year study. *Contact Dermatitis* 2000: 43: 181-182.

10. Frosch P J, Rastogi S C, Pirker C, Brinkmeier T, Andersen K E, Bruze M, Svedman C, Goossens A, White I R, Uter W, Arnau E G, Lepoittevin J P, Johansen J D, Menne T. Patch testing with a new fragrance mix - reactivity to the individual constituents and chemical detection in relevant cosmetic products. *Contact Dermatitis* 2005: 52: 216-225.

11. Krautheim A, Uter W, Frosch P, Schnuch A, Geier J. Patch testing with fragrance mix II: results of the IVDK 2005-2008. *Contact Dermatitis* 2010: 63: 262-269.

12. deGroot A C, Coenraads P J, Bruynzeel D P, Jagtman B A, van_Ginkel C J W, Noz K, van_der_Valk P G M, Pavel S, Vink J, Weyland J W. Routine patch testing with fragrance chemicals in The Netherlands. *Contact Dermatitis* 2000: 42: 184-185.

13. An S, Lee A Y, Lee C H, Kim D W, Hahm J H, Kim K J, Moon K C, Won Y H, Ro Y S, Eun H C. Fragrance contact dermatitis in Korea: a joint study. *Contact Dermatitis* 2005: 53: 320-323.

14. Sugiura M, Hayakawa R, Kato Y, Sugiura K, Hashimoto R. Results of patch testing with lavender oil in Japan. *Contact Dermatitis* 2000: 43: 157-160.

15. Frosch P J, Pilz B, Andersen K E, Burrows D, Camarasa J G, et al. Patch testing with fragrances: results of a multicenter study of the European Environmental and Contact Dermatitis Research Group with 48 frequently used constituents of perfumes. *Contact Dermatitis* 1995: 33: 333-342.

16. Frosch P J, Johansen J D, Menne T, Pirker C, Rastogi S C, Andersen K E, Bruze M, Goossens A, Lepoittevin J P, White I R. Further important sensitizers in patients sensitive to fragrances. I. Reactivity to 14 frequently used chemicals. *Contact Dermatitis* 2002: 47: 78-85.

17. Frosch P J, Johansen J D, Menne T, Pirker C, Rastogi S C, Andersen K E, Bruze M, Goossens A, Lepoittevin J P, White I R. Further important sensitizers in patients sensitive to fragrances. II. Reactivity to essential oils. *Contact Dermatitis* 2002: 47: 279-287.

18. Larsen W G. Perfume Dermatitis. A Study of 20 Patients. *Arch Dermatol* 1977: 113: 623-626.

19. Larsen W, Nakayama H, Fischer T, Elsner P, Frosch P, Burrows D, Jordan W, Shaw S, Wilkinson J, Marks J, Jr., Sugawara M, Nethercott M, Nethercott J. Fragrance contact dermatitis: a worldwide multicenter investigation (Part II). *Contact Dermatitis* 2001: 44: 344-346.

20. Belsito D V, Fowler J F, Jr., Sasseville D, Marks J G, Jr., De Leo V A, Storrs F J. Delayed-type hypersensitivity to fragrance materials in a select North American population. *Dermatitis* 2006: 17: 23-28.

21. Zug K A, Warshaw E M, Fowler J F, Jr., Maibach H I, Belsito D L, Pratt M D, Sasseville D, Storrs F J, Taylor J S, Mathias C G, Deleo V A, Rietschel R L, Marks J. Patch-test results of the North American Contact Dermatitis Group 2005-2006. *Dermatitis* 2009: 20: 149-160.

22. Wöhrl S, Hemmer W, Focke M, Götz M, Jarisch R. The significance of fragrance mix, balsam of Peru, colophony and propolis as screening tools in the detection of fragrance allergy. *Br J Dermatol* 2001: 145: 268-273.

23. Goossens A, Merckx L. Allergic Contact Dermatitis from farnesol in a deodorant. *Contact Dermatitis* 1997: 37: 179-180.

24. Malten K E, van Ketel W G, Nater J P, Liem D H. Reactions in selected patients to 22 fragrance materials. *Contact Dermatitis* 1984: 11: 1-10.

25. de Groot A C, Liem D H, Nater J P, van Ketel W G. Patch tests with fragrance materials and preservatives. *Contact Dermatitis* 1985: 12: 87-92.

26. Rudzki E, Grzywa Z, Bruo W S. Sensitivity to 35 essential oils. *Contact Dermatitis* 1976: 2: 196-200.

27. Rudzki E, Grzywa Z. Allergy to perfume mixture. *Contact Dermatitis* 1986: 15: 115-116.

28. Santucci B, Cristaudo A, Cannistraci C, Picardo M. Contact dermatitis to fragrances. *Contact Dermatitis* 1987: 16: 93-95.

29. Mitchell J C. Contact hypersensitivity to some perfume materials. *Contact Dermatitis* 1975: 1: 196-199.

30. Uter W, Schmidt E, Geier J, Lessmann H, Schnuch A, Frosch P J. Contact allergy to essential oils: current patch test results (2000-2008) from the IVDK network. *Contact Dermatitis* 2010: 63: 277-283.

31. Trattner A, David M. Patch testing with fine fragrances: comparison with fragrance mix, balsam of Peru and a fragrance series. *Contact Dermatitis* 2003: 49: 287-289.

32. Handley J, Burrows D. Allergic contact dermatitis from the synthetic fragrances Lyral and acetyl cedrene in separate underarm deodorant preparations. *Contact Dermatitis* 1994: 31: 288-290.

33. SCCNFP. The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers: Opinion concerning Fragrance Allergy in Consumers. A Review of the Problem. Analysis of the Need for appropriate Consumer Information and Identification of Consumer Allergens, adopted 8 December 1999. *SCCNFP/0017/98 Final* 1999:

34. Surburg H, Panten J. *Common fragrance and flavor materials: preparation, properties and uses*. Weinheim: Wiley-VCH, 2006.

35. Bhatia S P, Wellington G A, Cocchiara J, Lalko J, Letizia C S, Api A M. Fragrance material review on alpha-amylcinnamyl alcohol. *Food Chem Toxicol* 2007: 45 Suppl 1: S32-39.

36. Lapczynski A, McGinty D, Jones L, Bhatia S P, Letizia C S, Api A M. Fragrance material review on pentyl salicylate. *Food Chem Toxicol* 2007: 45 Suppl 1: S460-466.

37. Franks A. Contact allergy to anethole in toothpaste associated with loss of taste. *Contact Dermatitis* 1998: 38: 354-355.

38. Garcia-Bravo B, Perez Bernal A, Garcia-Hernandez M J, Camacho F. Occupational contact dermatitis from anethole in food handlers. *Contact Dermatitis* 1997: 37: 38.

39. Fahlbusch K-G, Hammerschmidt F-J, Panten J, Pickenhagen W, Schatkowski D, Bauer K, Garbe D, Surburg H. Flavors and Fragrances. In: Wiley-VCH, eds. *Ullmann's Encyclopedia of Industrial Chemistry*. Weinheim: Wiley-VCH, 2002:

40. Hostynek J J, Maibach H I. Is there evidence that anisyl alcohol causes allergic contact dermatitis? *Exog Dermatol* 2003: 2: 230-233.

40a. Bruze M, Svedman C, Andersen KE, Bruynzeel D, Goossens A, Johansen JD, Matura M, Orton D, Vigan M; ESCD. Patch test concentrations (doses in mg/cm2) for the 12 non-mix fragrance substances regulated by European legislation. *Contact Dermatitis* 2012: 66: 131-136

41. Andersen A. Final report on the safety assessment of benzaldehyde. *Int J Toxicol* 2006: 25 Suppl 1: 11-27.

42. Seite-Bellezza D, el Sayed F, Bazex J. Contact urticaria from cinnamic aldehyde and benzaldehyde in a confectioner. *Contact Dermatitis* 1994: 31: 272-273.

43. Opdyke D L, Letizia C. Monographs on fragrance raw materials. *Food Chem Toxicol* 1983: 21: 645-667.

44. Nair B. Final report on the safety assessment of Benzyl Alcohol, Benzoic Acid, and Sodium Benzoate. *Int J Toxicol* 2001: 20 Suppl 3: 23-50.

45. Sestini S, Mori M, Francalanci S. Allergic contact dermatitis from benzyl alcohol in multiple medicaments. *Contact Dermatitis* 2004: 50: 316-317.

46. Podda M, Zollner T, Grundmann-Kollmann M, Kaufmann R, Boehncke W H. Allergic contact dermatitis from benzyl alcohol during topical antimycotic treatment. *Contact Dermatitis* 1999: 41: 302-303.

47. Shoji A. Allergic reaction to benzyl alcohol in an antimycotic preparation. *Contact Dermatitis* 1983: 9: 510.

48. Cuesta L, Silvestre J F, Toledo F, Lucas A, Perez-Crespo M, Ballester I. Fragrance contact allergy: a 4-year retrospective study. *Contact Dermatitis* 2010: 63: 77-84.

49. Guin J D, Goodman J. Contact urticaria from benzyl alcohol presenting as intolerance to saline soaks. *Contact Dermatitis* 2001: 45: 182-183.

50. Fisher A A. Allergic paraben and benzyl alcohol hypersensitivity relationship of the "delayed" and "immediate" varieties. *Contact Dermatitis* 1975: 1: 281-284.

51. Shaw D W. Allergic contact dermatitis to benzyl alcohol in a hearing aid impression material. *Am J Contact Dermat* 1999: 10: 228-232.

52. Jacob S E, Barron G S. Benzyl alcohol: a covert fragrance. *Dermatitis* 2007: 18: 232-233.

53. Hausen B M, Brinkmann J, Dohn W. *Lexikon der Kontaktallergene (6. Erg.-Lieferung)*. Landsberg am Lech: Ecomed, 1998.

54. Corazza M, Manovani L, Maranini C, Virgili A. Allergic Contact Dermatitis from benzyl alcohol. *Contact Dermatitis* 1996: 34: 74.

55. Buffet M, Dupin N. Current treatments for scabies. *Fundam Clin Pharmacol* 2003: 17: 217-225.

56. Bhatia S P, Wellington G A, Cocchiara J, Lalko J, Letizia C S, Api A M. Fragrance material review on benzyl cinnamate. *Food Chem Toxicol* 2007: 45 Suppl 1: S40-48.

57. Lapczynski A, McGinty D, Jones L, Bhatia S, Letizia C S, Api A M. Fragrance material review on benzyl salicylate. *Food Chem Toxicol* 2007: 45 Suppl 1: S362-380.

58. Bhatia S P, Jones L, Letizia C S, Api A M. Fragrance material review on 2-tert-butylcyclohexyl acetate. *Food Chem Toxicol* 2008: 46 Suppl 12: S44-47.

59. Bhatia S P, Jones L, Letizia C S, Api A M. Fragrance material review on 4-tert-butylcyclohexyl acetate. *Food Chem Toxicol* 2008: 46 Suppl 12: S36-41.

60. Arnau E G, Andersen K E, Bruze M, Frosch P J, Johansen J D, Menne T, Rastogi S C, White I R, Lepoittevin J P. Identification of Lilial as a fragrance sensitizer in a perfume by bioassay-guided chemical fractionation and structure-activity relationships. *Contact Dermatitis* 2000: 43: 351-358.

61. Stevenson O E, Finch T M. Allergic contact dermatitis from rectified camphor oil in Earex ear drops. *Contact Dermatitis* 2003: 49: 51.

62. Vilaplana J, Romaguera C, Campderros L. [Contact dermatitis by camphor present in a flushing solution]. *Actas Dermosifiliogr* 2007: 98: 345-346.

63. Noiles K., M. P. Contact dermatitis to Vicks VapoRub. *Dermatitis* 2010: 21: 167-169.

64. Sköld M, Karlberg A T, Matura M, Börje A. The fragrance chemical beta-caryophyllene-air oxidation and skin sensitization. *Food Chem Toxicol* 2006: 44: 538-545.

65. Matura M, Skold M, Borje A, Andersen K E, Bruze M, Frosch P, Goossens A, Johansen J D, Svedman C, White I R, Karlberg A T. Selected oxidized fragrance terpenes are common contact allergens. *Contact Dermatitis* 2005: 52: 320-328.

66. Andersen A. Final report on the safety assessment of sodium p-chloro-m-cresol, p-chloro-m-cresol, chlorothymol, mixed cresols, m-cresol, o-cresol, p-cresol, isopropyl cresols, thymol, o-cymen-5-ol, and carvacrol. *Int J Toxicol* 2006: 25 Suppl 1: 29-127.

67. Corazza M, Levratti A, Virgili A. Allergic contact cheilitis due to carvone in toothpastes. *Contact Dermatitis* 2002: 46: 366-367.

68. Worm M, Jeep S, Sterry W, Zuberbier T. Perioral contact dermatitis caused by L-carvone in toothpaste. *Contact Dermatitis* 1998: 38: 338.

69. Hausen B M. Zahnpasta-Allergie. *Dtsch Med Wochenschr* 1984: 109: 300-302.

70. Paulsen E, Andersen K E, Carlsen L, et al. Carvone: an overlooked contact allergen cross-reacting with sesquiterpene lactones? *Contact Dermatitis* 1993: 29: 138-143.

71. Karlberg A T, Magnusson K, Nilsson U. Air oxidation of d-limonene (the citrus solvent) creates potent allergens. *Contact Dermatitis* 1992: 26: 332-340.

72. Matura M, Goossens A, Bordalo O, Garcia-Bravo B, Magnusson K, Wrangsjo K, Karlberg A T. Patch testing with oxidized R-(+)-limonene and its hydroperoxide fraction. *Contact Dermatitis* 2003: 49: 15-21.

73. Nilsson A M, Gafvert E, Salvador L, Luthman K, Bruze M, Gruvberger B, Nilsson J L, Karlberg A T. Mechanism of the antigen formation of carvone and related alpha, beta-unsaturated ketones. *Contact Dermatitis* 2001: 44: 347-356.

74. Nguyen S H, Dang T P, MacPherson C, Maibach H, Maibach H I. Prevalence of patch test results from 1970 to 2002 in a multi-centre population in North America (NACDG). *Contact Dermatitis* 2008: 58: 101-106.

75. Diba V C, Statham B N. Contact urticaria from cinnamal leading to anaphylaxis. *Contact Dermatitis* 2003: 48: 119.

76. Decapite T J, Anderson B E. Allergic contact dermatitis from cinnamic aldehyde found in an industrial odour-masking agent. *Contact Dermatitis* 2004: 51: 312-313.

77. Cocchiara J, Letizia C S, Lalko J, Lapczynski A, Api A M. Fragrance material review on cinnamaldehyde. *Food Chem Toxicol* 2005: 43: 867-923.

78. Bickers D, Calow P, Greim H, Hanifin J M, Rogers A E, Saurat J H, Sipes I G, Smith R L, Tagami H. A toxicologic and dermatologic assessment of cinnamyl alcohol, cinnamaldehyde and cinnamic acid when used as fragrance ingredients. *Food Chem Toxicol* 2005: 43: 799-836.

79. Buckley D A, Basketter D A, Smith Pease C K, Rycroft R J, White I R, McFadden J P. Simultaneous sensitivity to fragrances. *Br J Dermatol* 2006: 154: 885-888.

80. Elahi E N, Wright Z, Hinselwood D, Hotchkiss S A, Basketter D A, Pease C K. Protein binding and metabolism influence the relative skin sensitization potential of cinnamic compounds. *Chem Res Toxicol* 2004: 17: 301-310.

81. Letizia C S, Cocchiara J, Lalko J, Lapczynski A, Api A M. Fragrance material review on cinnamyl alcohol. *Food Chem Toxicol* 2005: 43: 837-866.

82. Heydorn S, Menne T, Andersen K E, Bruze M, Svedman C, White I R, Basketter D A. Citral a fragrance allergen and irritant. *Contact Dermatitis* 2003: 49: 32-36.

83. Hindle E, Ashworth J, Beck M H. Chelitis from contact allergy to citral in lip salve. *Contact Dermatitis* 2007: 57: 125-126.

84. Hagvall L, Backtorp C, Svensson S, Nyman G, Borje A, Karlberg A T. Fragrance compound geraniol forms contact allergens on air exposure. Identification and quantification of oxidation products and effect on skin sensitization. *Chem Res Toxicol* 2007: 20: 807-814.

85. Hagvall L, Baron J M, Borje A, Weidolf L, Merk H, Karlberg A T. Cytochrome P450-mediated activation of the fragrance compound geraniol forms potent contact allergens. *Toxicol Appl Pharmacol* 2008: 233: 308-313.

86. Lapczynski A, Bhatia S P, Letizia C S, Api A M. Fragrance material review on l-citronellol. *Food Chem Toxicol* 2008: 46 Suppl 11: S110-113.

87. Lapczynski A, Letizia C S, Api A M. Fragrance material review on (+)-(R)-citronellol. *Food Chem Toxicol* 2008: 46 Suppl 11: S114-116.

88. Lapczynski A, Bhatia S P, Letizia C S, Api A M. Fragrance material review on dl-citronellol. *Food Chem Toxicol* 2008: 46 Suppl 11: S103-109.

89. Hostynek J J, Maibach H I. Sensitization Potential of Citronellol. *Exog Dermatol* 2004: 3: 307-312.

90. Mutterer V, Gimenez Arnau E, Lepoittevin J P, Johansen J D, Frosch P J, Menne T, Andersen K E, Bruze M, Rastogi S C, White I R. Identification of coumarin as the sensitizer in a patient sensitive to her own perfume but negative to the fragrance mix. *Contact Dermatitis* 1999: 40: 196-199.

91. Vocanson M, Goujon C, Chabeau G, Castelain M, Valeyrie M, Floc'h F, Maliverney C, Gard A, Nicolas J F. The skin allergenic properties of chemicals may depend on contaminants--evidence from studies on coumarin. *Int Arch Allergy Immunol* 2006: 140: 231-238.

92. Bhatia S P, Letizia C S, Api A M. Fragrance material review on cyclohexyl acetate. *Food Chem Toxicol* 2008: 46 Suppl 12: S52-55.

93. Letizia C S, Cocchiara J, Wellington G A, Funk C, Api A M. Food and chemical toxicology. *Food Chem Toxicol* 2000: 38 Suppl 3: S1-236.

94. Lapczynski A, Lalko J, McGinty D, Bhatia S, Letizia C S, Api A M. Fragrance material review on damascenone. *Food Chem Toxicol* 2007: 45 Suppl 1: S172-178.

95. Takanami I, Nakayama H. TMCHB: a possible alternative to DNCB in skin testing for immune competence. *Contact Dermatitis* 1988: 19: 81-83.

96. Lapczynski A, Lalko J, McGinty D, Bhatia S, Letizia C S, Api A M. Fragrance material review on alpha-damascone. *Food Chem Toxicol* 2007: 45 Suppl 1: S179-187.

97. Lapczynski A, Lalko J, McGinty D, Bhatia S, Letizia C S, Api A M. Fragrance material review on cis-alpha-damascone. *Food Chem Toxicol* 2007: 45 Suppl 1: S188-191.

98. Lalko J, Lapczynski A, Letizia C S, Api A M. Fragrance material review on cis-beta-damascone. *Food Chem Toxicol* 2007: 45 Suppl 1: S192-198.

99. Lapczynski A, Lalko J, McGinty D, Bhatia S, Letizia C S, Api A M. Fragrance material review on trans-beta-damascone. *Food Chem Toxicol* 2007: 45 Suppl 1: S199-204.

100. Lalko J, Lapczynski A, McGinty D, Bhatia S, Letizia C S, Api A M. Fragrance material review on delta-damascone. *Food Chem Toxicol* 2007: 45 Suppl 1: S205-210.

101. Lapczynski A, Lalko J, McGinty D, Bhatia S, Letizia C S, Api A M. Fragrance material review on trans,trans-delta-damascone. *Food Chem Toxicol* 2007: 45 Suppl 1: S211-215.

102. Lalko J, Lapczynski A, McGinty D, Bhatiaa S, Letiziaa C S, Api A M. Fragrance material review on gamma-damascone. *Food Chem Toxicol* 2007: 45 (Suppl. 1): S216-S220.

103. McGinty D, Letizia C S, Api A M. Fragrance material review on dihydromyrcenol. *Food Chem Toxicol* 2010: 48 Suppl 3: S70-75.

104. McGinty D, Letizia C S, Api A M. Fragrance material review on 3,7-dimethyl-1,6-nonadien-3-ol. *Food Chem Toxicol* 2010: 48 Suppl 3: S52-55.

105. Mitchell D M, Beck M H. Contact allergy to benzyl alcohol in a cutting oil reodorant. *Contact Dermatitis* 1988: 18: 301-302.

106. Giusti F, Porcaro V, Seidenari S. Evaluation of eugenol allergy in a patch-test population. *Contact Dermatitis* 2001: 44: 37-38.

107. Quirce S, Fernandez-Nieto M, del Pozo V, Sastre B, Sastre J. Occupational asthma and rhinitis caused by eugenol in a hairdresser. *Allergy* 2008: 63: 137-138.

108. Bhalla M, Thami G P. Acute urticaria due to dental eugenol. *Allergy* 2003: 58: 158.

109. Sarrami N, Pemberton M N, Thornhill M H, Theaker E D. Adverse reactions associated with the use of eugenol in dentistry. *Br Dent J* 2002: 193: 257-259.

110. Kanerva L, Estlander T, Jolanki R. Dental nurse's occupational allergic contact dermatitis from eugenol used as a restorative dental material with polymethylmethacrylate. *Contact Dermatitis* 1998: 38: 339-340.

111. Hemmer W, Focke M, Leitner B, Gotz M, Jarisch R. Axillary dermatitis from farnesol in a deodorant. *Contact Dermatitis* 2000: 42: 168-169.

112. Schnuch A, Uter W, Geier J, Lessmann H, Frosch P J. Contact allergy to farnesol in 2021 consecutively patch tested patients. Results of the IVDK. *Contact Dermatitis* 2004: 50: 117-121.

113. Lapczynski A, Bhatia S P, Letizia C S, Api A M. Fragrance material review on farnesol. *Food Chem Toxicol* 2008: 46 Suppl 11: S149-156.

114. Tamagawa-Mineoka R, Katoh N, Kishimoto S. Allergic contact cheilitis due to geraniol in food. *Contact Dermatitis* 2007: 56: 242-243.

115. Yamamoto A, Morita A, Tsuji T, Suzuki K, Matsunaga K. Contact urticaria from geraniol. *Contact Dermatitis* 2002: 46: 52.

116. Hostynek J J, Maibach H I. Is there evidence that geraniol causes allergic contact dermatitis? *Exog Dermatol* 2004: 3: 318-331.

117. Lapczynski A, Bhatia S P, Foxenberg R J, Letizia C S, Api A M. Fragrance material review on geraniol. *Food Chem Toxicol* 2008: 46 Suppl 11: S160-170.

118. Dearman R J, Wright Z M, Basketter D A, Ryan C A, Gerberick G F, Kimber I. The suitability of hexyl cinnamic aldehyde as a calibrant for the murine local lymph node assay. *Contact Dermatitis* 2001: 44: 357-361.

119. Lapczynski A, Jones L, McGinty D, Bhatia S, Letizia C S, Api A M. Fragrance material review on hexyl salicylate. *Food Chem Toxicol* 2007: 45 Suppl 1: S410-417.

120. Karlberg A T. Contact allergy to colophony. Chemical identifications of allergens, sensitization experiments and clinical experiences. *Acta Dermatol Venerol (Stockh) Suppl* 1988: 139: 1-43.

121. Schnuch A, Uter W, Dickel H, Szliska C, Schliemann S, Eben R, Rueff F, Gimenez-Arnau A, Loffler H, Aberer W, Frambach Y, Worm M, Niebuhr M, Hillen U, Martin V, Jappe U, Frosch P J, Mahler V. Quantitative patch and repeated open application testing in hydroxyisohexyl 3-cyclohexene carboxaldehyde sensitive-patients. *Contact Dermatitis* 2009: 61: 152-162.

122. Hendriks S A, Bousema M T, van Ginkel C J. Allergic contact dermatitis from the fragrance ingredient Lyral in underarm deodorant. *Contact Dermatitis* 1999: 41: 119.

123. Lapczynski A, Bhatia S P, Letizia C S, Api A M. Fragrance material review on hydroxycitronellol. *Food Chem Toxicol* 2008: 46 Suppl 11: S179-181.

124. Lalko J, Lapczynski A, McGinty D, Bhatia S, Letizia C S, Api A M. Fragrance material review on ionone. *Food Chem Toxicol* 2007: 45 Suppl 1: S251-257.

125. Lalko J, Lapczynski A, Politano V T, McGinty D, Bhatia S, Letizia C S, Api A M. Fragrance material review on alpha-ionone. *Food Chem Toxicol* 2007: 45 Suppl 1: S235-240.

126. Lalko J, Lapczynski A, McGinty D, Bhatia S, Letizia C S, Api A M. Fragrance material review on beta-ionone. *Food Chem Toxicol* 2007: 45 Suppl 1: S241-247.

127. Lapczynski A, Jones L, McGinty D, Bhatia S, Letizia C S, Api A M. Fragrance material review on isoamyl salicylate. *Food Chem Toxicol* 2007: 45 Suppl 1: S418-423.

128. Ford R A, Api A M, Letizia C S. Monographs on fragrance raw materials. *Food Chem Toxicol* 1992: 30 Suppl: 1S-138S.

129. White J M, White I R, Glendinning A, Fleming J, Jefferies D, Basketter D A, McFadden J P, Buckley D A. Frequency of allergic contact dermatitis to isoeugenol is increasing: a review of 3636 patients tested from 2001 to 2005. *Br J Dermatol* 2007: 157: 580-582.

130. Tanaka S, Royds C, Buckley D, Basketter D A, Goossens A, Bruze M, Svedman C, Menne T, Johansen J D, White I R, McFadden J P. Contact allergy to isoeugenol and its derivatives: problems with allergen substitution. *Contact Dermatitis* 2004: 51: 288-291.

131. White I R, Johansen J D, Arnau E G, Lepoittevin J P, Rastogi S, Bruze M, Andersen K E, Frosch P J, Goossens A, Menne T. Isoeugenol is an important contact allergen: can it be safely replaced with isoeugenyl acetate? *Contact Dermatitis* 1999: 41: 272-275.

132. Lapczynski A, Lalko J, Politano V T, McGinty D, Bhatia S, Letizia C S, Api A M. Fragrance material review on alpha-iso-methylionone. *Food Chem Toxicol* 2007: 45 Suppl 1: S280-289.

133. Hostynek J J, Maibach H I. Is there evidence that alpha-isomethylionone causes allergic contact dermatitis? *Exog Dermatol* 2004: 3: 121-125.

134. Guarneri F, Barbuzza O, Vaccaro M, Galtieri G. Allergic contact dermatitis and asthma caused by limonene in a labourer handling citrus fruits. *Contact Dermatitis* 2008: 58: 315-316.

135. Foti C, Zambonin C G, Conserva A, Casulli C, D'Accolti L, Angelini G. Occupational contact dermatitis to a limonene-based solvent in a histopathology technician. *Contact Dermatitis* 2007: 56: 109-112.

136. Wakelin S H, McFadden J P, Leonard J N, Rycroft R J. Allergic contact dermatitis from d-limonene in a laboratory technician. *Contact Dermatitis* 1998: 38: 164-165.

137. Topham E J, Wakelin S H. D-Limonene contact dermatitis from hand cleansers. *Contact Dermatitis* 2003: 49: 108-109.

138. Martins C, Goncalo M, Goncalo S. Allergic contact dermatitis from dipentene in wax polish. *Contact Dermatitis* 1995: 33: 126-127.

139. Rycroft R J. Allergic contact dermatitis from dipentene in honing oil. *Contact Dermatitis* 1980: 6: 325-329.

140. Meding B, Barregard L, Marcus K. Hand eczema in car mechanics. *Contact Dermatitis* 1994: 30: 129-134.

141. Karlberg A T, Dooms-Gossens A. Contact allergy to oxidized d-limonene among dermatitis patients. *Contact Dermatitis* 1997: 36: 201-206.

142. Matura M, Skold M, Borje A, Andersen K E, Bruze M, Frosch P, Goossens A, Johansen J D, Svedman C, White I R, Karlberg A T. Not only oxidized R-(+)- but

also S-(-)-limonene is a common cause of contact allergy in dermatitis patients in Europe. *Contact Dermatitis* 2006: 55: 274-279.

143. Matura M, Goossens A, Bordalo O, Garcia-Bravo B, Magnusson K, Wrangsjo K, Karlberg A T. Oxidized citrus oil (R-limonene): a frequent skin sensitizer in Europe. *J Am Acad Dermatol* 2002: 47: 709-714.

144. Sköld M, Börje A, Harambasic E, Karlberg A T. Contact allergens formed on air exposure of linalool. Identification and quantification of primary and secondary oxidation products and the effect on skin sensitization. *Chem Res Toxicol* 2004: 17: 1697-1705.

145. Christensson J B, Matura M, Gruvberger B, Bruze M, Karlberg A T. Linalool--a significant contact sensitizer after air exposure. *Contact Dermatitis* 2010: 62: 32-41.

146. Letizia C S, Cocchiara J, Lalko J, Api A M. Fragrance material review on linalool. *Food Chem Toxicol* 2003: 41: 943-964.

147. Bickers D, Calow P, Greim H, Hanifin J M, Rogers A E, Saurat J H, Sipes I G, Smith R L, Tagami H. A toxicologic and dermatologic assessment of linalool and related esters when used as fragrance ingredients. *Food Chem Toxicol* 2003: 41: 919-942.

148. Hostynek J J, Maibach H I. Is there evidence that linalool causes allergic contact dermatitis? *Exog Dermatol* 2003: 2: 223-229.

149. de Groot A C, Bruynzeel D P, Bos J D, der Meeren H L v, van Joost T, Jagtman B A, Weyland J W. The allergens in cosmetics. *Arch Dermatol* 1988: 124: 1525-1529.

150. Sköld M, Hagvall L, Karlberg A T. Autoxidation of linalyl acetate, the main component of lavender oil, creates potent contact allergens. *Contact Dermatitis* 2008: 58: 9-14.

151. Hagvall L, Skold M, Brared-Christensson J, Borje A, Karlberg A T. Lavender oil lacks natural protection against autoxidation, forming strong contact allergens on air exposure. *Contact Dermatitis* 2008: 59: 143-150.

152. Letizia C S, Cocchiara J, Lalko J, Api A M. Fragrance material review on linalyl acetate. *Food Chem Toxicol* 2003: 41: 965-976.

153. Ford R A, Letizia C S, Api A M. Longifolene. *Food Chem Tox* 1992: 30(Suppl.): 67S-68S.

154. Morton C A, Garioch J, Todd P, et al. Contact sensitivity to menthol and peppermint in patients with intra-oral symptoms. *Contact Dermatitis* 1995: 32: 281-284.

155. Foti C, Conserva A, Antelmi A, Lospalluti L, Angelini G. Contact dermatitis from peppermint and menthol in a local action transcutaneous patch. *Contact Dermatitis* 2003: 49: 312-313.

156. Andersson M, Hindsen M. Rhinitis because of toothpaste and other menthol-containing products. *Allergy* 2007: 62: 336-337.

157. dos Santos M A, Santos Galvao C E, Morato Castro F. Menthol-induced asthma: a case report. *J Investig Allergol Clin Immunol* 2001: 11: 56-58.

158. Bhatia S P, McGinty D, Letizia C S, Api A M. Fragrance material review on menthol. *Food Chem Toxicol* 2008: 46 Suppl 11: S209-214.

159. Bhatia S P, McGinty D, Letizia C S, Api A M. Fragrance material review on d-menthol. *Food Chem Toxicol* 2008: 46 Suppl 11: S215-217.

160. Bhatia S P, McGinty D, Letizia C S, Api A M. Fragrance material review on l-menthol. *Food Chem Toxicol* 2008: 46 Suppl 11: S218-223.

161. Bhatia S P, McGinty D, Letizia C S, Api A M. Fragrance material review on d,l-menthol. *Food Chem Toxicol* 2008: 46 Suppl 11: S224-227.

162. Bhatia S P, McGinty D, Letizia C S, Api A M. Fragrance material review on menthol racemic. *Food Chem Toxicol* 2008: 46 Suppl 11: S228-233.

163. Nair B. 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. *Int J Toxicol* 2001: 20 Suppl 3: 61-73.

164. Trattner A, David M. Patch testing with fine fragrances: comparison with fragrance mix, balsam of Peru and a fragrance series. *Contact Dermatitis* 2003: 49: 287-289.

165. Mitchell J C, Calnan C D, Clendenning W E, Cronin E, Hjorth N, Magnusson B, Maibach H I, Meneghini C L, Wilkinson D S. Patch testing with some components of balsam of Peru. *Contact Dermatitis* 1976: 2: 57-58.

166. Bhatia S P, Wellington G A, Cocchiara J, Lalko J, Letizia C S, Api A M. Fragrance material review on methyl cinnamate. *Food Chem Toxicol* 2007: 45 Suppl 1: S113-119.

167. Kaidbey K H, Kligman A M. Photocontact allergy to 6-methylcoumarin. *Contact Dermatitis* 1978: 4: 277-282.

168. Cardoso J C, Canelas M M, Goncalo M, Figueiredo A. Photopatch testing with an extended series of photoallergens: a 5-year study. *Contact Dermatitis* 2009: 60: 325-329.

169. Victor F C, Cohen D E, Soter N A. A 20-year analysis of previous and emerging allergens that elicit photoallergic contact dermatitis. *J Am Acad Dermatol* 2010: 62: 605-610.

170. McGinty D, Letizia C S, Api A M. Fragrance material review on 4-methyl-3-decen-5-ol. *Food Chem Toxicol* 2010: 48 Suppl 3: S93-96.

171. Lalko J, Lapczynski A, McGinty D, Bhatia S, Letizia C S, Api A M. Fragrance material review on methyl ionone (mixture of isomers). *Food Chem Toxicol* 2007: 45 Suppl 1: S300-307.

172. English J S, Rycroft R J. Allergic contact dermatitis from methyl heptine and methyl octine carbonates. *Contact Dermatitis* 1988: 18: 174-175.

173. Hostynek J J, Maibach H I. Is there evidence that methyl heptine carbonate causes allergic contact dermatitis? *Cutan Ocul Toxicol* 2006: 25: 259-271.

174. Heisterberg M V, Vigan M, Johansen J D. Active sensitization and contact allergy to methyl 2-octynoate. *Contact Dermatitis* 2010: 62: 97-101.

175. Bernaola G, Escayol P, Fernandez E, de Corres L F. Contact dermatitis from methylionone fragrance. *Contact Dermatitis* 1989: 20: 71-72.

176. Lalko J, Lapczynski A, McGinty D, Bhatia S P, Letizia C S, Api A M. Fragrance material review on alpha-irone. *Food Chem Toxicol* 2007: 45 Suppl 1: S272-275.

177. Oiso N, Fukai K, Ishii M. Allergic contact dermatitis due to methyl salicylate in a compress. *Contact Dermatitis* 2004: 51: 34-35.

178. Hindson C. Contact eczema from methyl salicylate reproduced by oral aspirin (acetyl salicylic acid). *Contact Dermatitis* 1977: 3: 348-349.

179. Lapczynski A, Jones L, McGinty D, Bhatia S P, Letizia C S, Api A M. Fragrance material review on methyl salicylate. *Food Chem Toxicol* 2007: 45 Suppl 1: S428-452.

180. Sköld M. *Contact allergy to autoxidized fragrance terpenes*. Thesis University of Gothenburg 2005.

181. Bhatia S P, McGinty D, Letizia C S, Api A M. Fragrance material review on myrtenol. *Food Chem Toxicol* 2008: 46 Suppl 11: S237-240.

182. Lapczynski A, Foxenberg R J, Bhatia S P, Letizia C S, Api A M. Fragrance material review on nerol. *Food Chem Toxicol* 2008: 46 Suppl 11: S241-244.

183. Lapczynski A, Bhatia S P, Letizia C S, Api A M. Fragrance material review on nerolidol (isomer unspecified). *Food Chem Toxicol* 2008: 46 Suppl 11: S247-250.

184. Lapczynski A, Letizia C S, Api A M. Fragrance material review on cis-nerolidol. *Food Chem Toxicol* 2008: 46 Suppl 11: S245-246.

185. Christensen L P, Jakobsen H B, Paulsen E, Hodal L, Andersen K E. Airborne Compositae dermatitis: monoterpenes and no parthenolide are released from flowering Tanacetum parthenium (feverfew) plants. *Arch Dermatol Res* 1999: 291: 425-431.

186. Lapczynski A, McGinty D, Jones L, Bhatia S, Letizia C S, Api A M. Fragrance material review on phenethyl salicylate. *Food Chem Toxicol* 2007: 45 Suppl 1: S467-471.

187. Sanchez-Politta S, Campanelli A, Pashe-Koo F, Saurat J H, Piletta P. Allergic contact dermatitis to phenylacetaldehyde: a forgotten allergen? *Contact Dermatitis* 2007: 56: 171-172.

188. McGinty D, Letizia C S, Api A M. Fragrance material review on phytol. *Food Chem Toxicol* 2010: 48 Suppl 3: S59-63.

189. Cachao P, Menezes Brandao F, Carmo M, Frazao S, Silva M. Allergy to oil of turpentine in Portugal. *Contact Dermatitis* 1986: 14: 205-208.

190. Lear J T, Heagerty A H M, Tan B B, et al. Transient re-emergence of oil of turpentine allergy in the pottery industry. *Contact Dermatitis* 1996: 34: 169-172.

191. Zacher K D, Ippen H. Kontaktekzem durch Bergamottöl. *Derm Beruf Umwelt* 1984: 32: 95-97.

192. Lapczynski A, Bhatia S P, Letizia C S, Api A M. Fragrance material review on rhodinol. *Food Chem Toxicol* 2008: 46 Suppl 11: S259-262.

193. Lapczynski A, Lalko J, McGinty D, Bhatia S P, Letizia C S, Api A M. Fragrance material review on alpha-isodamascone. *Food Chem Toxicol* 2007: 45 Suppl 1: S267-271.

194. Bruze M, Zimerson E. Cross-reaction patterns in patients with contact allergy to simple methylol phenols. *Contact Dermatitis* 1997: 37: 82-86.

195. Barbaud A, Reichert-Penetrat S, Trechot P, Granel F, Schmutz J L. [Sensitization to resorcinol in a prescription verrucide preparation: unusual systemic clinical features and prevalence]. *Ann Dermatol Venereol* 2001: 128: 615-618.

196. Aalto-Korte K, Valimaa J, Henriks-Eckerman M L, Jolanki R. Allergic contact dermatitis from salicyl alcohol and salicylaldehyde in aspen bark (Populus tremula). *Contact Dermatitis* 2005: 52: 93-95.

197. Bhatia S P, McGinty D, Letizia C S, Api A M. Fragrance material review on alpha-santalol. *Food Chem Toxicol* 2008: 46 Suppl 11: S267-269.

198. Bhatia S P, McGinty D, Letizia C S, Api A M. Fragrance material review on santalol. *Food Chem Toxicol* 2008: 46 Suppl 11: S263-266.

199. Bhatia S P, McGinty D, Letizia C S, Api A M. Fragrance material review on sclareol. *Food Chem Toxicol* 2008: 46 Suppl 11: S270-274.

200. Bhatia S P, McGinty D, Foxenberg R J, Letizia C S, Api A M. Fragrance material review on terpineol. *Food Chem Toxicol* 2008: 46 Suppl 11: S275-279.

201. Bhatia S P, Letizia C S, Api A M. Fragrance material review on (-)-alpha-terpineol. *Food Chem Toxicol* 2008: 46 Suppl 11: S204-205.

202. Bhatia S P, Letizia C S, Api A M. Fragrance material review on alpha-terpineol. *Food Chem Toxicol* 2008: 46 Suppl 11: S280-285.

203. Castelain P Y, Camoin J P, Jouglard J. Contact dermatitis to terpene derivatives in a machine cleaner. *Contact Dermatitis* 1980: 6: 358-360.

204. Hausen B M, Reichling J, Harkenthal M. Degradation products of monoterpenes are the sensitizing agents in tea tree oil. *Am J Contact Dermat* 1999: 10: 68-77.

205. Lapczynski A, Foxenberg R J, Bhatia S P, Letizia C S, Api A M. Fragrance material review on tetrahydrolinalool. *Food Chem Toxicol* 2008: 46 Suppl 11: S286-288.

206. Schnuch A, Geier J, Uter W, Frosch P J. Majantol--a new important fragrance allergen. *Contact Dermatitis* 2007: 57: 48-50.

207. Heisterberg M V, Johansen J D. Contact allergy to trimethyl-benzenepropanol (Majantol). *Contact Dermatitis* 2009: 61: 360-361.

208. Hausen B M. Contact allergy to balsam of Peru. II. Patch test results in 102 patients with selected balsam of Peru constituents. *Am J Contact Dermat* 2001: 12: 93-102.

209. Rudzki E, Grzywa Z. Dermatitis from propolis. *Contact Dermatitis* 1983: 9: 40-45.

210. Ferguson J E, Beck M H. Contact sensitivity to vanilla in a lip salve. *Contact Dermatitis* 1995: 33: 352.

211. Bhatia S P, Letizia C S, Api A M. Fragrance material review on tricyclo[5.2.1.02,6]dec-4-en-8-yl acetate. *Food Chem Toxicol* 2008: 46 Suppl 12: S100-101.

212. Bhatia S P, Jones L, Letizia C S, Api A M. Fragrance material review on tricyclodecenyl acetate. *Food Chem Toxicol* 2008: 46 Suppl 12: S93-96.

213. Anonymous. *ISO/DIS 9235 Aromatic raw materials - vocabulary*. Geneva, Switzerland: International Standardisation Organisation, 2010.

214. Schmidt E. Production of Essential Oils. In: Husnu Can Baser K, Buchbauer G, eds. *Handbook of Essential Oils - Science, Technology, and Applications*. Boca Raton: CRC Press, 2010: 88-95.

215. Trattner A, David M, Lazarov A. Occupational contact dermatitis due to essential oils. *Contact Dermatitis* 2008: 58: 282-284.

216. Jung P, Sesztak-Greinecker G, Wantke F, Gotz M, Jarisch R, Hemmer W. Mechanical irritation triggering allergic contact dermatitis from essential oils in a masseur. *Contact Dermatitis* 2006: 54: 297-299.

217. Bilsland D, Strong A. Allergic contact dermatitis from the essential oil of French marigold (Tagetes patula) in an aromatherapist. *Contact Dermatitis* 1990: 23: 55-56.

218. Cockayne S E, Gawkrodger D J. Occupational contact dermatitis in an aromatherapist. *Contact Dermatitis* 1997: 37: 306-307.

219. Boonchai W, Iamtharachai P, Sunthonpalin P. Occupational allergic contact dermatitis from essential oils in aromatherapists. *Contact Dermatitis* 2007: 56: 181-182.

220. Keane F M, Smith H R, White I R, Rycroft R J. Occupational allergic contact dermatitis in two aromatherapists. *Contact Dermatitis* 2000: 43: 49-51.

221. Selvaag E, Holm J O, Thune P. Allergic contact dermatitis in an aroma therapist with multiple sensitizations to essential oils. *Contact Dermatitis* 1995: 33: 354-355.

222. Romaguera C, Vilaplana J. Occupational contact dermatitis from ylang-ylang oil. *Contact Dermatitis* 2000: 43: 251.

223. Sugawara M, Nakayama H, Watanabe S. Contact hypersensitivity to ylang-ylang oil. *Contact Dermatitis* 1990: 23: 248-249.

224. Kanerva L, Estlander T, Jolanki R. Occupational allergic contact dermatitis caused by ylang-ylang oil. *Contact Dermatitis* 1995: 33: 198-199.

225. Sanchez-Perez J, Garcia-Diez A. Occupational allergic contact dermatitis from eugenol, oil of cinnamon and oil of cloves in a physiotherapist. *Contact Dermatitis* 1999: 41: 346-347.

226. Vilaplana J, Romaguera C. Contact dermatitis from the essential oil of tangerine in fragrance. *Contact Dermatitis* 2002: 46: 108.

227. Lalko J, Api A M. Investigation of the dermal sensitization potential of various essential oils in the local lymph node assay. *Food Chem Toxicol* 2006: 44: 739-746.

228. Vilaplana J, Romaguera C. Allergic contact dermatitis due to eucalyptol in an anti-inflammatory cream. *Contact Dermatitis* 2000: 43: 118.

229. Commission M. *List of MAK and BAT Values 2010 (Report No. 46)*. Weinheim: Wiley-VCH, 2011.

230. Nardelli A, Gimenez-Arnau E, Bernard G, Lepoittevin J P, Goossens A. Is a low content in atranol/chloroatranol safe in oak moss-sensitized individuals? *Contact Dermatitis* 2009: 60: 91-95.

231. Johansen J D, Heydorn S, Menne T. Oak moss extracts in the diagnosis of fragrance contact allergy. *Contact Dermatitis* 2002: 46: 157-161.

232. Kanerva L, Jolanki R, Estlander T. Hairdresser's dermatitis caused by oak moss in permanent waving solution. *Contact Dermatitis* 1999: 41: 55-56.

233. Owen C M, August P J, Beck M H. Contact allergy to oak moss resin in a soluble oil. *Contact Dermatitis* 2000: 43: 112.

234. Rudzki E, Grzywa Z. Sensitizing and irritating properties of star anise oil. *Contact Dermatitis* 1976: 2: 305-308.

235. Franz H, Frank R, Rytter M, Haustein U F. Allergic contact dermatitis due to cedarwood oil after dermatoscopy. *Contact Dermatitis* 1998: 38: 182-183.

236. Adisen E, Önder M. Allergic contact dermatitis from Laurus nobilis oil induced by massage. *Contact Dermatitis* 2007: 56: 360-361.

237. Athanasiadis G I, Pfab F, Klein A, Braun-Falco M, Ring J, Ollert M. Erythema multiforme due to contact with laurel oil. *Contact Dermatitis* 2007: 57: 116-118.

238. Özden M G, Öztas P, Öztas M O, Önder M. Allergic contact dermatitis from Laurus nobilis (laurel) oil. *Contact Dermatitis* 2001: 45: 178.

239. Goiriz R, Delgado-Jimenez Y, Sanchez-Perez J, Garcia-Diez A. Photoallergic contact dermatitis from lavender oil in topical ketoprofen. *Contact Dermatitis* 2007: 57: 381-382.

240. Rademaker M. Allergic contact dermatitis from lavender fragrance in Difflam gel. *Contact Dermatitis* 1994: 31:

241. Varma S, Blackford S, Statham B N, Blackwell A. Combined contact allergy to tea tree oil and lavender oil complicating chronic vulvovaginitis. *Contact Dermatitis* 2000: 42: 309-310.

242. Coulson I H, Khan A S. Facial 'pillow' dermatitis due to lavender oil allergy. *Contact Dermatitis* 1999: 41: 111.

243. Vermaat H, van Meurs T, Rustemeyer T, Bruynzeel D P, Kirtschig G. Vulval allergic contact dermatitis due to peppermint oil in herbal tea. *Contact Dermatitis* 2008: 58: 364-365.

244. Kalavala M, Hughes T M, Goodwin R G, Anstey A V, Stone N M. Allergic contact dermatitis to peppermint foot spray. *Contact Dermatitis* 2007: 57: 57-58.

245. Wilkinson S M, Beck M H. Allergic contact dermatitis from menthol in peppermint. *Contact Dermatitis* 1994: 30: 42.

246. Andersen K E. Contact allergy to toothpaste flavors. *Contact Dermatitis* 1978: 4: 195-198.

247. Clayton R, Orton D. Contact allergy to spearmint oil in a patient with oral lichen planus. *Contact Dermatitis* 2004: 51: 314-315.

248. Skrebova N, Brocks K, Karlsmark T. Allergic contact cheilitis from spearmint oil. *Contact Dermatitis* 1998: 39: 35.

249. Hänsel R, Keller K, Rimpler H, Schneider G. *Hagers Handbuch der pharmazeutischen Praxis. Drogen E - O*. Berlin, 894-902: Springer, 1993.

250. Hausen B M, Wollenweber E. Propolis allergy. (III). Sensitization studies with minor constituents. *Contact Dermatitis* 1988: 19: 296-303.

251. Hausen, M B, Evers, P, Stüwe, T H, et al. Propolis allergy (IV) Studies with further sensitizers from propolis and constituents common to propolis, poplar buds and balsam of Peru. *Contact Dermatitis* 1992: 26: 34-44.

252. Hausen B M, Simatupang T, Bruhn G, Evers P, König W A. Identification of new allergenic constituents and proof of evidence for coniferyl benzoate in Balsam of Peru. *Am J Contact Dermat* 1995: 6: 199-208.

253. Hjorth N. Eczematous allergy to balsams, allied perfumes and flavouring agents. *Acta Derm Venereol* 1961: 41 (Suppl. 46): 1-216.

254. Wurm G. *Hagers Handbuch der pharmazeutischen Praxis. Waren und Dienste*. Berlin, 644-689: Springer, 1990.

255. Api A M. Only Peru Balsam extracts or distillates are used in perfumery. *Contact Dermatitis* 2006: 54: 179.

256. Temesvari E, Podanyi B, Ponyai G, Nemeth I. Fragrance sensitization caused by temporary henna tattoo. *Contact Dermatitis* 2002: 47: 240.

257. Lammintausta K, Maibach H I, Wilson D. Mechanisms of subjective (sensory) irritation. Propensity to non- immunologic contact urticaria and objective irritation in stingers. *Derm Beruf Umwelt* 1988: 36: 45-49.

258. Forsbeck M, Skog E. Immediate reactions to patch tests with balsam of Peru. *Contact Dermatitis* 1977: 3: 201-205.

259. Katsarou A, Armenaka M, Ale I, Koufou V, Kalogeromitros D. Frequency of immediate reactions to the European standard series. *Contact Dermatitis* 1999: 41: 276-279.

260. Temesvari E, Soos G, Podanyi B, Kovacs I, Nemeth I. Contact urticaria provoked by balsam of Peru. *Contact Dermatitis* 1978: 4: 65-68.

261. Cancian M, Fortina A B, Peserico A. Contact urticaria syndrome from constituents of balsam of Peru and fragrance mix in a patient with chronic urticaria. *Contact Dermatitis* 1999: 41: 300.

262. Tanaka S, Matsumoto Y, Dlova N, Ostlere L S, Goldsmith P C, Rycroft R J, Basketter D A, White I R, Banerjee P, McFadden J P. Immediate contact reactions to fragrance mix constituents and Myroxylon pereirae resin. *Contact Dermatitis* 2004: 51: 20-21.

263. Uter W, Lessmann H. Kontaktallergene. In: Schulze-Werninghaus G, Fuchs T, Bachert C, Wahn U, eds. *Manuale allergologicum*. Deisenhofen: Dustri, 2008: 237-308.

264. Freireich-Astman M, David M, Trattner A. Standard patch test results in patients with contact dermatitis in Israel: age and sex differences. *Contact Dermatitis* 2007: 56: 103-107.

265. Lazarov A. European Standard Series patch test results from a contact dermatitis clinic in Israel during the 7-year period from 1998 to 2004. *Contact Dermatitis* 2006: 55: 73-76.

266. Gupta N, Shenoi S D, Balachandran C. Fragrance sensitivity in allergic contact dermatitis. *Contact Dermatitis* 1999: 40: 53-54.

267. Kashani M N, Gorouhi F, Behnia F, Nazemi M J, Dowlati Y, Firooz A. Allergic contact dermatitis in Iran. *Contact Dermatitis* 2005: 52: 154-158.

268. Avalos-Peralta P, Garcia-Bravo B, Camacho F M. Sensitivity to Myroxylon pereirae resin (balsam of Peru). A study of 50 cases. *Contact Dermatitis* 2005: 52: 304-306.

269. Akyol A, Boyvat A, Peksari Y, Gurgey E. Contact sensitivity to standard series allergens in 1038 patients with contact dermatitis in Turkey. *Contact Dermatitis* 2005: 52: 333-337.

270. Machovcova A, Dastychova E, Kostalova D, Vojtechovska A, Reslova J, Smejkalova D, Vaneckova J, Vocilkova A. Common contact sensitizers in the Czech Republic. Patch test results in 12,058 patients with suspected contact dermatitis*. *Contact Dermatitis* 2005: 53: 162-166.

271. Thyssen J P, Carlsen B C, Menne T, Johansen J D. Trends of contact allergy to fragrance mix I and Myroxylon pereirae among Danish eczema patients tested between 1985 and 2007. *Contact Dermatitis* 2008: 59: 238-244.

272. Lindberg M, Edman B, Fischer T, Stenberg B. Time trends in Swedish patch test data from 1992 to 2000. A multi-centre study based on age- and sex-adjusted results of the Swedish standard series. *Contact Dermatitis* 2007: 56: 205-210.

273. Uter W, Hegewald J, Aberer W, Ayala F, Bircher A J, Brasch J, Coenraads P J, Schuttelaar M L, Elsner P, Fartasch M, Mahler V, Belloni Fortina A, Frosch P J, Fuchs T, Johansen J D, Menne T, Jolanki R, Krecisz B, Kiec-Swierczynska M, Larese F, Orton D, Peserico A, Rantanen T, Schnuch A. The European standard series in 9 European countries, 2002/2003 - First results of the European Surveillance System on Contact Allergies. *Contact Dermatitis* 2005: 53: 136-145.

274. Bruynzeel D P, Diepgen T L, Andersen K E, Brandao F M, Bruze M, Frosch P J, Goossens A, Lahti A, Mahler V, Maibach H I, Menne T, Wilkinson J D. Monitoring

the European standard series in 10 centres 1996-2000. *Contact Dermatitis* 2005: 53: 146-149.

275. Vilaplana J, Romaguera C, Grimalt F. Contact dermatitis from geraniol in Bulgarian rose oil. *Contact Dermatitis* 1991: 24: 301.

276. Nardelli A, Thijs L, Janssen K, Goossens A. Rosa centifolia in a 'non-scented' moisturizing body lotion as a cause of allergic contact dermatitis. *Contact Dermatitis* 2009: 61: 306-309.

277. Howes M J, Simmonds M S, Kite G C. Evaluation of the quality of sandalwood essential oils by gas chromatography-mass spectrometry. *J Chromatogr A* 2004: 1028: 307-312.

278. Burdock G A, Carabin I G. Safety assessment of sandalwood oil (Santalum album L.). *Food Chem Toxicol* 2008: 46: 421-432.

279. Treudler R, Richter G, Geier J, Schnuch A, Orfanos C E, Tebbe B. Increase in sensitization to oil of turpentine: recent data from a multicenter study on 45,005 patients from the German-Austrian Information Network of Departments of Dermatology (IVDK). *Contact Dermatitis* 2000: 42: 68-73.

280. Schnuch A, Lessmann H, Geier J, Frosch P J, Uter W. Contact allergy to fragrances: frequencies of sensitization from 1996 to 2002. Results of the IVDK*. *Contact Dermatitis* 2004: 50: 65-76.

281. Schäfer T, Böhler E, Ruhdorfer S, Weigl L, Wessner D, Filipiak B, Wichmann H E, Ring J. Epidemiology of contact allergy in adults. *Allergy* 2001: 56: 1192-1196.

Annex II - Animal Data

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

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

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

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
p-tert-Butyl-dihydrocinnamaldehyde	18127-01-0	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	4.3	1075	0.23	4.3		RIFM, 2007b
Butylphenyl methylpropional (BMHCA)	80-54-6	EtOH	1.0, 3.0, 10.0, 30.0, 50.0	4	2.9	725	0.14	2.9		RIFM, 2001a
Butylphenyl methylpropional (BMHCA)	80-54-6	DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	4.1	1025	0.20			RIFM, 2001b
Butylphenyl methylpropional (BMHCA)	80-54-6	1:3 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	13.9	3475	0.68			RIFM, 2001c
Butylphenyl methylpropional (BMHCA)	80-54-6	1:3 DEP:EtOH	0.3, 1.0, 3.0, 10.0, 30.0	4	8.8	2200	0.43			RIFM, 2001d
Butylphenyl methylpropional (BMHCA)	80-54-6	4:1 AOO	1.0, 2.5, 5.0, 10.0, 25.0	4	16.8	4200	0.82			RIFM, 2001e

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

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

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

		EtOH:DEP							
Cinnamal	104-55-2	0.1% Trolox C in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	0.7	175	0.053		RIFM, 2003e
Cinnamal	104-55-2	2.0% α-tocopherol in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	0.8	200	0.060		RIFM, 2003f
Cinnamal	104-55-2	3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	0.9	225	0.068		RIFM, 2003g
Cinnamal	104-55-2	0.1% α-tocopherol in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	1.1	275	0.083		RIFM, 2003h
Cinnamal	104-55-2	0.3% antioxidant mix (equal parts BHT, tocopherol and eugenol) in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	1.3	325	0.098		RIFM, 2003i
Cinnamal	104-55-2	0.1% Trolox C in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	1.4	350	0.11		RIFM, 2003j
Cinnamal	104-55-2	-	-	-	-	-	-	No data in the ref (poster abstract)	Basketter et al., 2002
Cinnamal	104-55-2	4:1 AOO	0.5, 1, 2.5, 5, 10	4	3.1	775	0.23		Basketter et al., 2001
Cinnamal	104-55-2	4:1 AOO	-	4	1.3	325	0.10		Elahi et al., 2004
Cinnamal	104-55-2	4:1 AOO	1, 2.5	-	1.4	348	0.11	Too few concentrations tested; few details given in ref	Smith and Hotchkiss, 2001
Cinnamal	104-55-2	4:1 AOO	1.0, 2.5, 5.0, 10.0, 25.0	4	1.7	425	0.13		Wright et al., 1995

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

Cinnamal	104-55-2	MEK	1.0, 2.5, 5.0, 10.0, 25.0	4	1.1	275	0.083			Wright et al., 1996
Cinnamal	104-55-2	DMF	0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0	4	0.5	125	0.038			Wright et al., 1997
Cinnamal	104-55-2	propylene glycol	1.0, 2.5, 5.0, 10.0, 25.0	4	1.4	350	0.11			Wright et al., 1998
Cinnamal	104-55-2	DMSO	0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0	4	0.9	225	0.068			Wright et al., 1999
Cinnamal	104-55-2	90:10 EtOH:water	1.0, 2.5, 5.0, 10.0, 25.0	4	1.6	400	0.12			Wright et al., 2000
Cinnamal	104-55-2	50:50 EtOH:water	1.0, 2.5, 5.0, 10.0, 25.0	4	1.2	300	0.091			Wright et al., 2001
Cinnamyl alcohol	104-54-1	-	-	-	-	-	-		No data in the ref (poster abstract)	Basketter et al., 2002
Cinnamyl nitrile	1885-38-7	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	> 10	>2500	>0.77	> 10	Report: systemic toxicity at 25% and 50%. Should have been tested at higher concentrations	RIFM, 2005f
Citral	5392-40-5	1:3 EtOH:DEP	0.4, 2.0, 4.0, 8.0, 20.0	4	1.2	300	0.079	1.2		RIFM, 2004b
Citral	5392-40-5	0.1% α-tocopherol in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	1.5	375	0.099			RIFM, 2003k

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

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

Coumarin	91-64-5	DMF	10, 25, 50	4	>50	>12500	>3.42	>50	Should have been tested at higher concentrations	Vocanson et al., 2006
Dibenzyl ether	103-50-4	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	6.3	1575	0.32	6.3		RIFM, 2007e
Eugenol	97-53-0	3:1 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	5.3	1325	0.32	5.3		RIFM, 2001f
Eugenol	97-53-0	1:3 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	10.5	2625	0.64			RIFM, 2001g
Eugenol	97-53-0	EtOH	1.0, 3.0, 10.0, 30.0, 50.0	4	10.7	2675	0.65			RIFM, 2001h
Eugenol	97-53-0	DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	15.1	3775	0.92			RIFM, 2001i
Eugenol	97-53-0	4:1 AOO	2.5, 5.0, 10.0, 25.0, 50.0	-	11.9	2975	0.72			Basketter et al., 1999
Eugenol	97-53-0	-	-	-	-	-	-		No data in the ref (poster abstract)	Basketter et al., 2003
Evernia furfuracea extract *Treemoss absolute*	90028-67-4	1:3 EtOH:DEP	5.0, 10.0, 20	4	> 20	>5000	N/a	> 20	Should have been tested at higher concentrations	RIFM, 2004k
Evernia furfuracea extract *Treemoss absolute*	90028-67-4	1:3 EtOH:DEP	10.0, 25.0	4	> 25	>6250	N/a		Too few concentrations tested	RIFM, 2004d

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

Evernia prunastri extract *Oakmoss*	90028-68-5	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	3.88	970	N/a	3.88		RIFM, 2004j
Farnesol	4602-84-0	4:1 AOO	5.0, 10.0, 25.0	4	5.5	1375	0.25		Should also have been tested at lower concentrations	RIFM, 2004d
Farnesol	4602-84-0	4:1 AOO	5.0, 10.0, 25.0	4	4.1	1025	0.18	4.1	Should also have been tested at lower concentrations	RIFM, 2004d
Geraniol	106-24-1	EtOH	1.0, 3.0, 10.0, 30.0, 50.0	4	5.6	1400	0.36	5.6		RIFM, 2001j
Geraniol	106-24-1	3:1 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	11.4	2850	0.74			RIFM, 2003t
Geraniol	106-24-1	DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	11.8	2950	0.76			RIFM, 2001k
Geraniol	106-24-1	1:3 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	20.4	5100	1.32			RIFM, 2001l
Geraniol	106-24-1	3:1 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	25.8	6450	1.67			RIFM, 2001m
Geraniol	106-24-1	-	-	-	26	6500	1.69			Roberts et al., 2007
trans-2-Hexenal	6728-26-3	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5, 10	4	2.6	650	0.26	2.6		RIFM, 2005g
trans-2-Hexenal	6728-26-3	-	-	-	5.5	1375	0.56			Roberts et al., 2007

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

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

Hydroxycitronellal	107-75-5	4:1 AOO	10, 25	-	23	5750	1.34		Too few concentrations tested; few details given in ref	Smith and Hotchkiss, 2001
Hydroxyisohexyl 3-cyclohexene carboxaldehyde	31906-04-4	4:1 AOO	1.0, 2.5, 5, 10, 25, 50	4	17.1	4275	0.81	17.1		RIFM, 2001r
p-Isobutyl-α-methyl hydrocinnamaldehdye	6658-48-6	70% EtOH	10.0, 25.0, 50.0, 100.0	4	9.5	2375	0.46	9.5	Should also have been tested at lower concentrations	RIFM, 2001w
Isocyclocitral	1335-66-6	1:3 EtOH:DEP	0.5 , 1.0 , 2.5 , 5.0, 10.0	4	7.3	1825	0.48	7.3		RIFM, 2006c
Isocyclogeraniol	68527-77-5	1:3 EtOH:DEP	5.0, 10.0, 25.0, 50.0	4	> 25	>6250	>1.62	> 25	Should have been tested at higher concentrations	RIFM, 2005h
Isoeugenol	97-54-1	4:1 AOO	0.5, 5.0	6	0.54	145	0.033	0.54	Too few concentrations tested	RIFM, 2001s
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	0.6	150	0.037			RIFM, 2002a
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	0.76	191	0.046			RIFM, 2002b
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	0.79	199	0.048			RIFM, 2002c

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	4:1 AOO	0.5, 1.0, 5.0	5	1.19	296	0.072		RIFM, 2001t
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	1.28	320	0.078		RIFM, 2004e
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	6	1.54	385	0.094		RIFM, 2001u
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	1.95	488	0.119		RIFM, 2001v
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0		3.3	825	0.20		Basketter et al., 1999
Isoeugenol	97-54-1	-	-	-	-	-	-	No data in the ref (poster abstract)	Basketter et al., 2002
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	1.3	325	0.079		Loveless et al., 1996
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	3.3	825	0.20		Loveless et al., 1996
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	1.8	450	0.11		Loveless et al., 1996
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	3.1	775	0.19		Loveless et al., 1996
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	1.6	400	0.097		Loveless et al., 1996
Isoeugenol	97-54-1	AOO	0.5, 1.0, 2.5, 5.0, 10.0	4	1.0	250	0.061		Wright et al., 2001
Isoeugenol	97-54-1	MEK	0.5, 1.0, 2.5, 5.0, 10.0	4	1.0	250	0.061		Wright et al., 2001
Isoeugenol	97-54-1	DMF	0.5, 1.0, 2.5, 5.0, 10.0	4	1.4	350	0.085		Wright et al., 2001

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

Isoeugenol	97-54-1	propylene glycol	0.5, 1.0, 2.5, 5.0, 10.0	4	2.5	625	0.15			Wright et al., 2001
Isoeugenol	97-54-1	DMSO	0.5, 1.0, 2.5, 5.0, 10.0	4	0.9	225	0.055			Wright et al., 2001
Isoeugenol	97-54-1	90:10 EtOH:water	0.5, 1.0, 2.5, 5.0, 10.0	4	1.8	450	0.11			Wright et al., 2001
Isoeugenol	97-54-1	50:50 EtOH:water	0.5, 1.0, 2.5, 5.0, 10.0	4	4.9	1225	0.30			Wright et al., 2001
Jasmine absolute (Grandiflorum)	8022-96-6	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	5.9	1475	N/a	5.9		RIFM, 2006d
Jasminum Sambac Flower CERA / Extract / Water	91770-14-8	1:3 EtOH:DEP	10.0, 25.0, 50.0, 75.0, 100.0	4	35.4	9100	N/a	35.4		RIFM, 2006e
*d-Limonene***	5989-27-5	EtOH	10.0, 20.0, 50.0, 75.0, 100.0	4	< 10	< 250	<0.73	< 10	Should also have been tested at lower concentrations	RIFM, 2004l
*d-Limonene***	5989-27-5	3:1 EtOH:DEP	10.0, 20.0, 50.0, 75.0, 100.0	4	22.0	5500	1.61			RIFM, 2004m
*d-Limonene***	5989-27-5	1:3 EtOH:DEP	10.0, 20.0, 50.0, 75.0, 100.0	4	38.0	9500	2.79			RIFM, 2004n
*d-Limonene***	5989-27-5	DEP	10.0, 20.0, 50.0, 75.0, 100.0	4	63.0	15.75	4.62			RIFM, 2004o
*d-Limonene***	5989-27-5	4:1 AOO	25, 50, 100	4	68.5	17125	5.03			Warbrick et al., 2001

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

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

Methyl 2-octynoate	111-12-6	1:3 EtOH:DEP	0.5, 1.0, 2.0, 5.0, 10.0	4	< 0.5	< 125	<0.032	< 0.5	Should also have been tested at lower concentrations	RIFM, 2005k
2-Methoxy-4-methylphenol	93-51-6	-	-	-	5.8	1450	0.42	5.8		Roberts et al., 2007
1-Octen-3-yl acetate	2442-10-6	1:3 EtOH:DEP	7.5, 15.0, 30.0	5	> 30	> 7500	>1.76	> 30	Should have been tested at higher concentrations	RIFM, 2004g
Perillaldehyde *p-Mentha-1,8-dien-7-al*	2111-75-3	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	5	9.3	2325	0.62			RIFM, 2008b
Perillaldehyde *p-Mentha-1,8-dien-7-al*	2111-75-3	-	-	-	8.1	2025	0.54	8.1		Roberts et al., 2007
Balsam oil, Peru (Myroxylon pereirae Klotzsch)	8007-00-9	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	3.95	987	N/a	3.95		RIFM, 2004h
Peru balsam absolute	8007-00-9	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	2.5	625	N/a	2.5		RIFM, 2004i
Peru balsam absolute	8007-00-9	1:3 EtOH:DEP	0.5, 1.0, 2.5	4	>2.5	>625	N/a			RIFM, 2004i
Phenylacetaldehyde	122-78-1	4:1 AOO	2.5, 5, 10, 25, 50	4	3.0	750	0.25	3.0		Basketter et al., 2001

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

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

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

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

- = no data given; A216

References

Aptula, N., Roberts, D.W., Schultz, T.W., Pease, C., 2007. Reactivity assays for non-animal based prediction of skin sensitisation potential. Toxicology, 231(2-3), 117-118

Ashby J, Basketter D.A., Patton, D., Kimber I. 1995. Structure activity relationships in skin sensitization using the murine local lymph node assay. Toxicology 103:177-194

Basketter, D.A., Gilmour, N., Dearman, R.J., Kimber, I., Ryan, C.A., Gerberick, F., 2003. Classification of skin sensitisation potency using the Local Lymph Node Assay. The Toxicologist, 72(S-1), 101

Basketter, D. A., Lea, L. J., Dickens, A., Briggs, D., Pate, I., Dearman, R. J., Kimber, I., 1999. A comparison of statistical approaches to the derivation of EC3 values from local lymph node assay dose responses. Journal of Applied Toxicology, 19(4), 261-266

Basketter, D.A., Wright, Z., Gilmour, N.J., Ryan, C.A., Gerberick, G.F., Robinson, M.K., Dearman, R.J., Kimber, I., 2002. Prediction of human sensitization potency using local lymph node assay EC3 values. The Toxicologist, 66(1-S), 240

Basketter, D. A., Wright, Z. M., Warbrick, E. V., Dearman, R. J., Kimber, I., Ryan, C. A., Gerberick, G. F., White, I. R., 2001. Human potency predictions for aldehydes using the local lymph node assay. Contact Dermatitis, 45(2), 89-94

Elahi, E.N., Wright, Z., Hinselwood, D., Hotchkiss, S.A.M., Basketter, D.A., Smith Pease, C.K., 2004. Protein binding and metabolism influence the relative skin sensitization potential of cinnamic compounds. Chemical Research in Toxicology, 17(3), 301-310

Estrada, E., Patlewicz, G., Chamberlain, M., Basketter, D., Larbey, S., 2003. Computer aided Knowledge Generation for Understanding Skin Sensitization Mechanisms: The TOPS-MODE Approach. Chem. Res. Toxicol., 16, 1226-1235

Gerberick, G.F., Ryan, C.A, . Kern, P.S., Dearman, R.J., Kimber, I., Patlewicz, G.Y., Basketter, D.A. 2004. A chemical dataset for evaluation of alternative approaches to skin-sensitization testing. Contact Dermatitis 50, 274-288

Loveless, S. E., Ladics, G. S., Gerberick, G. F., Ryan, C. A., Basketter, D. A., Scholes, E. W., House, R. V., Hilton, J., Dearman, R. J., Kimber, I., 1996. Further evaluation of the local lymph node assay in the final phase of an international collaborative trial. Toxicology, 108(1-2), 141-152

Nilsson, A.-M., Bergstrom, M.A., Luthman, K., Nilsson, J.L.G., Karlberg, A.-T., 2005. An alpha,beta-unsaturated oxime identified as a strong contact allergen. Indications of antigen formation via several pathways. Food and Chemical Toxicology, 43(11), 1627-1636

RIFM, 2001a. Local Lymph Node Assay on p-t-Butyl-α-methyl-hydrocinnamic aldehyde in EtOH . RIFM report number 37065 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001b. Local Lymph Node Assay on p-t-Butyl-α-methyl-hydrocinnamic aldehyde in DEP. RIFM report number 37066 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001c. Local Lymph Node Assay on p-t-Butyl-α-methyl-hydrocinnamic aldehyde in 1:3 EtOH:DEP. RIFM report number 37067 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001d. Local Lymph Node Assay on p-t-Butyl-α-methyl-hydrocinnamic aldehyde in 1:3 DEP:EtOH. RIFM report number 37068 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001e. Local Lymph Node Assay on p-t-Butyl-α-methyl-hydrocinnamic aldehyde in 4:1 acetone:olive oil. RIFM report number 41235. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001f. Local Lymph Node Assay on eugenol. RIFM report number 37076. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001g. Local Lymph Node Assay on eugenol. RIFM report number 37075. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001h. Local Lymph Node Assay on eugenol. RIFM report number 37073. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001i. Local Lymph Node Assay on eugenol. RIFM report number 37074. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001j. Local Lymph Node Assay on geraniol in ethanol. RIFM report number 37069 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001k. Local Lymph Node Assay on geraniol in DEP. RIFM report number 37070 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001l. Local Lymph Node Assay on geraniol in 1:3 EtOH:DEP. RIFM report number 37071 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001m. Local Lymph Node Assay on geraniol in 3:1 EtOH:DEP. RIFM report number 37072 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001n. Local Lymph Node Assay on hydroxycitronellal in 1:3 EtOH:DEP. RIFM report number 37079 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001o. Local Lymph Node Assay on hydroxycitronellal in DEP. RIFM report number 37078 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001p. Local Lymph Node Assay on hydroxycitronellal in 3:1 EtOH:DEP. RIFM report number 37080 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001q. Local Lymph Node Assay on hydroxycitronellal in EtOH. RIFM report number 37080 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001r. Local Lymph Node Assay on p-t-Butyl-α-methyl-hydrocinnamic aldehyde in 4:1 acetone:olive oil. RIFM report number 41235. Unpublished report from Unilever. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001s. Local Lymph Node Assay on isoeugenol in 4:1 acetone:olive oil. RIFM report number 59516. Unpublished report from Firmenich. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001t. Local Lymph Node Assay on isoeugenol in 4:1 acetone:olive oil. RIFM report number 42122. Unpublished report from Firmenich. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001u. Local Lymph Node Assay on isoeugenol in 4:1 acetone:olive oil. RIFM report number 40676. Unpublished report from Firmenich. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001v. Local Lymph Node Assay on isoeugenol in 4:1 acetone:olive oil. RIFM report number 42120. Unpublished report from Firmenich. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001w. Local Lymph Node Assay on p-isobutyl-α-methyl hydrocinnamaldehdye in 70% Ethanol. RIFM report number 41055. Unpublished report from Givaudan. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2002a. Local Lymph Node Assay on isoeugenol in 4:1 acetone:olive oil. RIFM report number 42139. Unpublished report from Firmenich. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2002b. Local Lymph Node Assay on isoeugenol in 4:1 acetone:olive oil. RIFM report number 42145. Unpublished report from Firmenich. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2002c. Local Lymph Node Assay on isoeugenol in 4:1 acetone:olive oil. RIFM report number 42123. Unpublished report from Firmenich. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2002d. Local Lymph Node Assay on majantol in 4:1 acetone:olive oil. RIFM report number 58693. Unpublished report from Symrise. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003a. Local Lymph Node Assay on cinnamic aldehyde in 3:1 EtOH:DEP. RIFM report number 42032 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003b. Local Lymph Node Assay on cinnamic aldehyde in 3:1 EtOH:DEP with 0.1% tocopherol. RIFM report number 42033 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003c. Local Lymph Node Assay on cinnamic aldehyde in 3:1 EtOH:DEP with 2.0% tocopherol. RIFM report number 42040 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003d. Local Lymph Node Assay on cinnamic aldehyde in 3:1 EtOH:DEP with antioxidant mix. RIFM report number 42034 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003e. Local Lymph Node Assay on cinnamic aldehyde in 3:1 EtOH:DEP with 0.1% Trolox C. RIFM report number 42036 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003f. Local Lymph Node Assay on cinnamic aldehyde in 3:1 EtOH:DEP with 2.0% tocopherol. RIFM report number 42035 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003g. Local Lymph Node Assay on cinnamic aldehyde in 3:1 EtOH:DEP. RIFM report number 42037 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003h. Local Lymph Node Assay on cinnamic aldehyde in 3:1 EtOH:DEP with 0.1% tocopherol. RIFM report number 42038 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003i. Local Lymph Node Assay on cinnamic aldehyde in 3:1 EtOH:DEP with antioxidant mix. RIFM report number 42039 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003j. Local Lymph Node Assay on cinnamic aldehyde in 3:1 EtOH:DEP with 0.1% Trolox C. RIFM report number 42041 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003k. Local Lymph Node Assay on citral in 3:1 EtOH:DEP with 0.1% tocopherol. RIFM report number 42028 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003l. Local Lymph Node Assay on citral in 3:1 EtOH:DEP with antioxidant mix. RIFM report number 42025 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003m. Local Lymph Node Assay on citral in 3:1 EtOH:DEP with antioxidant mix. RIFM report number 42026 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003n. Local Lymph Node Assay on citral in 3:1 EtOH:DEP. RIFM report number 42023 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003o. Local Lymph Node Assay on citral in 3:1 EtOH:DEP with antioxidant mix. RIFM report number 42029 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003p. Local Lymph Node Assay on citral in 3:1 EtOH:DEP with antioxidant mix. RIFM report number 42027 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003q. Local Lymph Node Assay on citral in 3:1 EtOH:DEP with 0.1% Trolox C. RIFM report number 42030 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003r. Local Lymph Node Assay on citral. RIFM report number 43822 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003s. Local Lymph Node Assay on citral. RIFM report number 42024 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003t. Local Lymph Node Assay on geraniol in 3:1 EtOH:DEP. RIFM report number 43812 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2004a. Local Lymph Node Assay on α-amylcinnamyl alcohol. RIFM report number 45128 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2004b. Local Lymph Node Assay on Citral. RIFM report number 45126 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2004c. Local Lymph Node Assay on d,l-Citronellol. RIFM report number 48752 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2004d. Local Lymph Node Assay on farnesol RIFM report number 47136. Unpublished report from Symrise (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2004e. Local Lymph Node Assay on isoeugenol RIFM report number 47326. Unpublished report from Firmenich (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2004f. Local Lymph Node Assay on 4-methoxy-α-methyl benzenpropanal. RIFM report number 47809. Unpublished report from IFF (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2004g. Local Lymph Node Assay on 1-Octen-3-yl acetate. RIFM report number 47809. Unpublished report from IFF (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2004h. Local Lymph Node Assay on Peru Balsam Oil. RIFM report number 44372. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2004i. Local Lymph Node Assay on Peru Balsam Absolute. RIFM report number 44371. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2004j. Oakmoss absolute: Local lymph node assay. RIFM report number 43861 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2004k. Treemoss absolute: Local lymph node assay. RIFM report number 44368 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2004l. d-limonene: Local lymph node assay. RIFM report number 45756 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2004m. d-limonene: Local lymph node assay. RIFM report number 45753 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2004n. d-limonene: Local lymph node assay. RIFM report number 45755 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2004o. d-limonene: Local lymph node assay. RIFM report number 45754 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2005a. Local Lymph Node Assay on anisyl alcohol. RIFM report number 45755 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2005b. Local Lymph Node Assay on benzyl alcohol. RIFM report number 47376 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2005c. Local Lymph Node Assay on benzyl benzoate. RIFM report number 47377 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2005d. Local Lymph Node Assay on benzyl cinnamate. RIFM report number 48751 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2005e. Local Lymph Node Assay on benzyl salicylate. RIFM report number 47378 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2005f. Local Lymph Node Assay on cinnamyl nitrile. RIFM report number 51626 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2005g. Local Lymph Node Assay on trans-2-hexenal in 1:3 EtOH:DEP. RIFM report number 48756 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2005h. Local Lymph Node Assay on isocyclogeraniol in 1:3 EtOH:DEP. RIFM report number 48755 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2005i. Local Lymph Node Assay on α-Methyl-1,3-benzodioxole- 5-propionaldehyde in 1:3 EtOH:DEP. RIFM report number 50886 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2005j. Local Lymph Node Assay on α-iso-Methylionone in 1:3 EtOH:DEP. RIFM report number 48749 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2005k. Local Lymph Node Assay on Methyl 2-octynoate in 1:3 EtOH:DEP. RIFM report number 48753 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2005l. Local Lymph Node Assay on OTNE in 1:3 EtOH:DEP. RIFM report number 51630 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2005m. Local Lymph Node Assay on tea leaf absolute. RIFM report number 47597. Unpublished report from Robertet (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2006a. Local Lymph Node Assay on α-amylcinnamaldehyde. RIFM report number 52888 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2006b. Local Lymph Node Assay on hexyl salicylate. RIFM report number 51636 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2006c. Local Lymph Node Assay on isocyclocitral. RIFM report number 52892 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2006d. Local Lymph Node Assay on Jasmine Absolute (Grandiflorum). RIFM report number 53024 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2006e. Local Lymph Node Assay on Jasmine Absolute (Sambac). RIFM report number 52885 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2007b. Local Lymph Node Assay on p-t-Butyl-dihydrocinnamaldehyde. RIFM report number 52900 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2007c. Local Lymph Node Assay on carvone. RIFM report number 52902 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2007d. Local Lymph Node Assay on carvone. RIFM report number 52907 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2007e. Local Lymph Node Assay on dibenzyl ether. RIFM report number 52901 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2007f. Local Lymph Node Assay on Ylang Ylang Extra. RIFM report number 52903 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2008a. Local Lymph Node Assay on 2-hexylidene cyclopentanone. RIFM report number 55548 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2008b. Local Lymph Node Assay on p-mentha-1,8-dien-7-al. RIFM report number 54428 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2008c. Local Lymph Node Assay on menthadiene-7-methyl formate. RIFM report number 54429 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2008d. Local Lymph Node Assay on 6-methyl-3,5-heptadien-2-one. RIFM report number 55564 (RIFM, Woodcliff Lake, NJ, USA)

RIFM. 2009. Research Institute for Fragrance Materials, Inc. Local lymph node assay (LLNA) protocol summaries: Data presented at the 46th Congress of the European Societies of Toxicology

Roberts, D.W., Patlewicz, G., Kern, P.S., Gerberick, F., Kimber, I., Dearman, R.J., Ryan, C.A., Basketter, D.A., Aptula, A.O., 2007. Mechanistic applicability domain classification of a local lymph node assay dataset for skin sensitization. Chemical Research in Toxicology, 20(7), 1019-1030

Smith, C.K., Hotchkiss, S.A.M., 2001. Allergic Contact Dermatitis. Taylor & Francis, New York

Vocanson, M., Goujon, C., Chabeau, G., Castelain, M., Valeyrie, M., Floch, F, Maliverney, C., Gard A., Nicolas, J.F 2006. The skin allergenic properties of chemicals may depend on contaminants - evidence from studies on coumarin. International Archives of Allergy and Immunology, 140, 231-238

Warbrick, E.V.R., Dearman J., Ashby J., Schmezer P. and Kimber I. 2001. Preliminary assessment of the skin sensitizing activity of selected rodent carcinogens using the local lymph node assay. Toxicology, 163(1), 63-69

Wright, Z. M., Basketter, D. A., Blaikie, L., Cooper, K. J., Warbrick, E. V., Dearman, R. J., Kimber, I., 2001. Vehicle effects on skin sensitization potency of four chemicals assessment using the local lymph node assay. International Journal of Cosmetic Science, 23(2), 75-83

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

Contents

Chloroatranol

Chloroatranol (allergen in oak moss absolute: *Evernia prunastri*) (1)	
Design	blinded, randomised with regard to doses and controlled
Test subjects	13 patients previously identified as sensitized to chloroatranol and oak moss absolute
Controls	10 healthy controls
Substance	Purity: >99%
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	200 ppm to 0.0063 ppm (10 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction
ROAT	volar aspect of forearms
area	3 x 3 cm^2
applications/day	two
dose	chloroatranol in ethanol: Step 1: 5 ppm Step 2: 25 ppm
dose/application/cm^2	step 1: 0.025 µg step2: 0.125 µg
control substance	ethanol
definition of positive	erythema in at least 25% and at least one papule
period	two weeks for each step
Results	
PT ED10% (95% CI)	0.013 (0.002-0.03) ppm =0.0004 µg/cm^2
PT ED50% (95% CI)	0.15 (0.077-0.295) ppm =0.0045 µg/cm^2
PT no effect level (observed)	/
ROAT	Cumulative responses
Step 1 (5 ppm)	12/13 (92%)
Step 2 (25 ppm)	13/13 (100%)
Controls	Negative
Other information	None relevant

In a subsequent study chloroatranol and atranol, both ingredients in *Evernia prunastri*, were tested in equimolar concentrations in serial dilution in 10 eczema patients with known sensitization to chloroatranol and oak moss. A positive response was defined as any degree of reaction. Ethanol was included as the control and gave no response. No use tests were done and no control subjects included.

Results: All patients reacted to the highest concentrations of the two substances. For both substances there was a significant dose-dependence and the estimated difference in elicitation potency of chloroatranol relative to atranol was 217%. The dose-response curve is seen in figure 1 below (2).

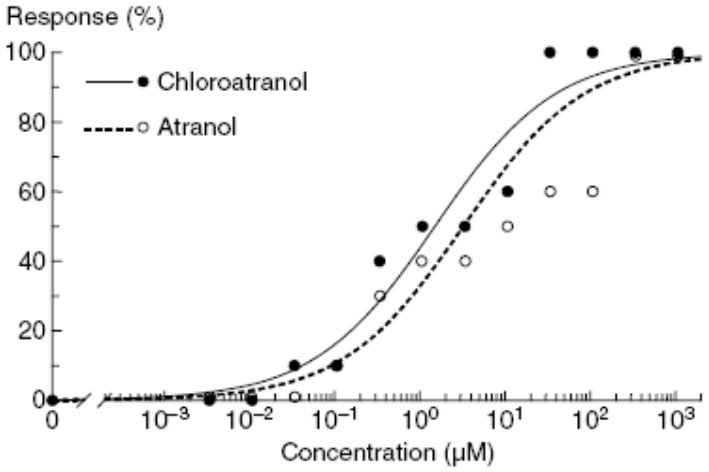


Fig. 1. Observed response rates and fitted parallel logistic dose–response curves for atranol and chloroatranol in equimolar concentrations at patch testing. The response was dichotomized and any reaction other than zero was classified as positive.

Cinnamal

Cinnamal (3)	
Design	blinded, randomised and controlled
Test subjects	18 patients with a positive patch test to cinnamal and additional 4 with a doubtful response
Controls	20 healthy controls
Substance	Purity: >98%
Patch test	20 mg solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2% to 0.01% (7 steps)
-control/vehicle	petrolatum
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of responses
ROAT	outer aspect of upper arm
area	5 x 5 cm^2
applications/day	two with atomizer pump
dose	Step 1: 0.02% Step 2: 0.1% Step 3: 0.8%
dose/application/cm^2	Not given
control substance	ethanol
definition of positive	The response was classified as positive no matter the degree of reaction.
period	two weeks for each step; total maximum 6 weeks
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	0.24% = 96 µg/cm^2 (calculated from the data in the paper)
PT no effect level(observed)	0.01 % in pet. = 0.4 µg/cm^2
ROAT	Cumulative responses
Step 1 (0.02%)	0/18
Step 2 (0.1%)	8/18 (44 %)
Step 3 (0.8%)	13/18 (72 %)
Controls	No eczema reactions were seen
Other information	2 patients and 2 controls developed immediate reactions to the cinnamal solution

Cinnamal *(4)*	
Design	blinded, randomised doses and controlled
Test subjects	17 patients with a positive patch test to cinnamal (8 patients in part 1 and 9 in part two)
Controls	20 controls (non-sensitised dermatitis patients)
Substance	purity: /
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2 % to 0.00006 % (17 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration eliciting a + reaction
ROAT	Axilla
area	10 x 10 cm^2 (estimated)
applications/day	two with roll on deodorant (89-700 mg per application of solution) average cases: 263 mg/application controls: only range given
dose	Part one: Step 1: 0.032% Step 2: 0.1% Step: 0.32% Part two: Step 1: 0.01% Step 2: 0.032% Step 3: 0.1%
dose/application/cm^2	Part two estimated: step one: 0.26 µg; step two: 0.84 µg; 2.63 µg
control substance	Deodorant matrix
definition of positive	eczematous reaction covering at least 25% of test area
period	Part one: one week with each concentration: maximum three weeks Part two: two weeks with each concentration: maximum six weeks
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	/
PT no effect level(observed)	0.002%
ROAT	Cumulative responses
Step 1 (0.01)	2/9 (22%)
Step 2 (0.032)	6/9 (67%)
Step 3 (0.1)	8/9 (88%)
Controls	No reactions were seen
Other information	Only reactions seen to the cinnamal-containing deodorants at ROAT, difference to matrix axilla ($p<0.001$) and all control

	persons negative ($p<0.001$)

Hydroxycitronellal

Hydroxycitronellal *(5)*	
Design	blinded, randomised doses and controlled
Test subjects	7 patients with a positive patch test to hydroxycitronellal
Controls	7 controls (non-sensitised dermatitis patients)
Substance	purity: /
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	4% to 0.00006% (17 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration eliciting + reaction
ROAT	Axilla
area	10 x 10 cm^2 (estimated)
applications/day	two with roll on deodorant (172-591 per application of solution) average cases: 294 mg/application controls: only range given
dose	Step 1: 0.032% Step 2: 0.1% Step: 0.32%
dose/application/cm^2	Estimated: step 1: 0.94 µg; step 2: 2.94 µg; step 3: 9.40 µg
control substance	Deodorant matrix
definition of positive	eczematous reaction covering at least 25% of test area
period	two weeks with each concentration: maximum six weeks
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	/
PT no effect level(observed)	<0.00012 %
ROAT	Cumulative responses
Step 1 (0.032)	4/7 (57%)
Step 2 (0.1)	5/7 (71%)
Step 3 (0.32)	7/7 (100%)
Controls	No reactions were seen
Other information	Reactions were only seen to the hydroxycitronellal-containing deodorant at ROAT, difference to matrix treated axilla (p<0.001) and all control persons negative (p<0.001)

Hydroxycitronellal (6)	
Design	double blinded, randomised
Test subjects	13 patients with a positive patch test to hydroxycitronellal
Controls	/
Substance	purity: unknown
Patch test	confirmatory
-dilution steps	
-control/vehicle	
-definition of threshold	
ROAT	finger immersion in fragrance solution in 10% ethanol
area	/
applications/day	Once per day for 10 min
dose	Step 1: 10 ppm Step 2: 250 ppm
dose/application/cm^2	Not applicable
control substance	10% alcohol
definition of positive	clinical grading scale and laser doppler comparison between active and control
period	two weeks with each concentration: maximum four weeks
Results	
PT ED10% (95% CI)	Not relevant
PT ED50% (95% CI)	Not relevant
PT no effect level(observed)	Not relevant
ROAT	Cumulative responses
Step 1 (10 ppm)	1/13
Step 2 (250 ppm)	5/13
Vehicle control	4/13
Other information	No difference between active substance and control application was found.

Hydroxyisohexyl 3-cyclohexenecarboxaldehyde (HICC)

Hydroxyisohexyl 3-cyclohexenecarboxaldehyde (HICC) *(7)*	
Design	blinded, randomised and controlled
Test subjects	18 patients with a positive patch test to HICC
Controls	7 healthy controls
Substance	Purity: >99%
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	6% to 0.0006%
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of reactions
ROAT	volar aspect of lower arm
area	3 x 3 cm^2
applications/day	two with droplet bottle (theoretical:30 mg per application of solution)
dose	Step 1: 0.5% Step 2: 3%
µg/application/cm^2	Step 1: 15.3 (3.4-22.2) Step 2: 126.2 (40.5-226.2)
control substance	ethanol
definition of positive	erythema in at least 25% and at least one papule
period	two weeks for each step; total maximum 4 weeks
Results	
PT ED10% (95% CI)	0.9 µg/cm^2 29 (7-69) ppm
PT ED50% (95% CI)	20 µg/cm^2 662 (350-1250)ppm
PT no effect level (observed)	/
ROAT	Cumulative responses
Step 1 (0.5%)	11/18 (61%)
Step 2 (3%)	16/18 (89%)
Controls	No reactions were seen
Other information	Difference between test and control group statistically significant

Hydroxyisohexyl 3-cyclohexenecarboxaldehyde (HICC) *(8)*	
Design	blinded, randomised and controlled
Test subjects	15 patients with a positive patch test to HICC
Controls	10 healthy controls
Substance	Purity: > 98.8%
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	6% to 0.0006% (5 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of reactions
ROAT	Axilla
area	76 cm^2 (template)
applications/day	two with roll on deodorant
dose	Step 1: 200 ppm Step 2: 600 ppm Step 3: 1800 ppm
dose/application/cm^2	median 0.79 µg HICC
control substance	deodorant matrix
definition of positive	spotty erythema involving at least 25% of the exposed area and infiltration represented by at least one papule.
period	two weeks for each step; total maximum 6 weeks
Results	
PT ED10% (95% CI)	0.75 µg/cm^2 25 ppm (0.69-120)
PT ED50% (95% CI)	18.3 µg/cm^2 610 ppm (120-2800)
PT no effect level (observed)	< 0.0006%
ROAT	Cumulative responses
Step 1 (200 ppm)	9/14* (64%)
Step 2 (600 ppm)	12/14* (86%)
Step 3 (1800 ppm)	14/14* (100%)
Controls	No reactions were seen
Other information	*14 patients completed the use test study Difference between HICC deodorant and matrix deodorant in cases (p=0.0001).Difference between controls and patients (p=0.004).

Hydroxyisohexyl 3-cyclohexenecarboxaldehyde (HICC) *(9)*	
Design	blinded, randomised and controlled
Test subjects	17 patients with a positive patch test to HICC
Controls	15 healthy controls
Substance	IFF lot SM/8059062
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	1500 to 0.0022 µg/cm^2 HICC (19 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of reactions to higher concentrations
ROAT	volar aspect of forearms
area	3 x 3 cm (5 areas)
applications/day	two with micropipette (20 µl per application)
dose	Simultaneous application to 5 areas, four doses each and vehicle
µg /application/cm^2	Dose 1:0.0357 Dose 2: 0.357 Dose 3: 3.57 Dose 4: 35.7
control substance	ethanol
definition of positive	at least 5 points on a clinical scale, corresponding to erythema in 25% of test area and at least 1 papule
period	Three weeks. All concentrations applied simultaneously (randomised)
Results	
PT ED10% (95% CI)	0.662 µg/ cm^2 (0.052-2.35)
PT ED50% (95% CI)	11.1 µg/ cm^2 (3.41- 33.1)
PT no effect level(observed)	<0.0022 µg/ cm^2
ROAT	Cumulative responses
Dose 1 (0.0357)	0/16*
Dose 2 (0.357)	3/16 (19%)
Dose 3 (3.57)	12/16 (75%)
Dose 4 (35.7)	15/16 (94%)
Controls	No reactions were seen
Other information	*16 patients completed the use test study The evaporation rate of HICC was calculated to 72% over a 24-h period. ED10% ROAT: 0.064 µg/cm^2 (more info see below)

Table 2 The dose per application and accumulated dose after 1, 2 and 3 weeks in the ROAT

ROAT, dose per application (μg HICC cm^{-2})	Number of applications after 1 week	Total accumulated dose after 1 week (μg HICC cm^{-2})	Number of applications after 2 weeks	Total accumulated dose after 2 weeks (μg HICC cm^{-2})	Number of applications after 3 weeks	Total accumulated dose after 3 weeks (μg HICC cm^{-2})
35·7	14	500	28	1000	42	1500
3·57	14	50	28	100	42	150
0·357	14	5	28	10	42	15
0·0357	14	0·5	28	1	42	1·5

ROAT, repeated open application test; HICC, hydroxyisohexyl-3-cyclohexene carboxaldehyde.

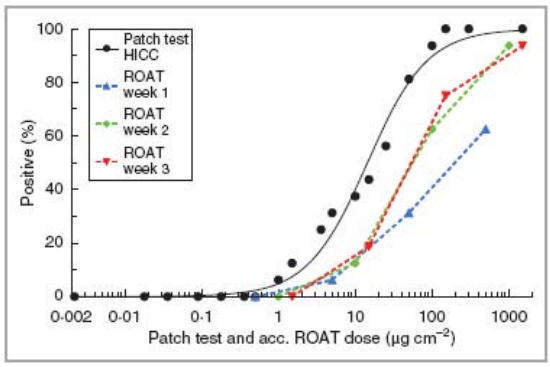


Fig 3. The fitted dose–response curve for the patch test (n = 16) and the 1-week, the 2-week 3-week accumulated ROAT doses.

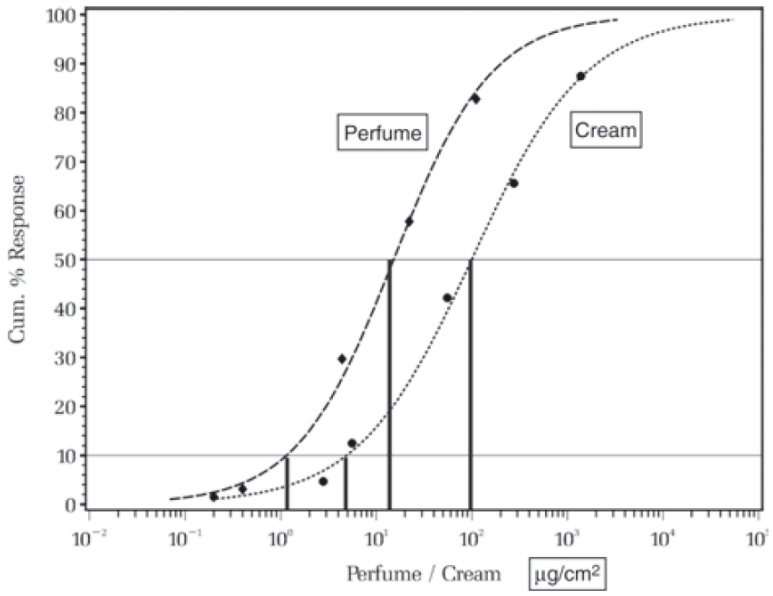
Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)

In a study be the German Contact Dermatitis Group, 64 persons previously diagnosed with HICC contact allergy were exposed to increasing doses of HICC in 2 different formulations, a hydrophilic cream and an ethanol solution, to mimic everyday exposures, following a standardised ROAT protocol (10). The concentration of HICC tolerated by 90% of the sensitised was estimated as 1.2 µg/cm² for perfume and 4.9 µg/cm² for cream. The dose-response curve is shown in Fig. 4.3 – 1 below.

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) (10)		
Design	randomised and vehicle controlled	
Test subjects	67 patients with a previous positive patch test to HICC	
Controls	None	
Substance	Provided by International Flavor & Fragrances Inc, Hilversum, NL	
Patch test		
-dilution steps	2.5% and 5%	
-control/vehicle	petrolatum	
-definition of threshold	lowest concentration giving a positive skin reaction in a continuous line to next higher concentration.	
ROAT	Volar forearms (both sides)	
area	3 x 3 cm (4 areas: one test and one control each for alcoholic solution and cream, respectively)	
applications/day	two	
dose	2.8 µg/cm² in cream 5.6 µg/cm² in cream 55.6 µg/cm² in cream 277.8 µg/cm² in cream 1388.9 µg/cm² in cream	0.2 µg/cm² in ethanol 0.4 µg/cm² in ethanol 4.4 µg/cm² in ethanol 22.2 µg/cm² in ethanol 111.1 µg/cm² in ethanol
µg /application/cm^2	See above	
control substance	Ethanol 96% and glyceryl stearate 15% in water, resp.	
definition of positive	(spotty) erythema of at least 25% of the test area along with homogeneous infiltration or papules regardless of the number	
period	Two weeks for each step until positive reaction or end of study, whichever occurred first	
Results		
PT ED10% (95% CI)	Not calculable; 52 of 60 Patients patch tested positive to 2.5% HICC, 57 / 60 to 5% HICC	
PT ED50% (95% CI)	Not calculable	
PT no effect level (observed)	Not calculable	
ROAT	Cumulative responses:	
	Cream preparation: 2.8 µg/cm²: 4.7%	Ethanol preparation: 0.2 µg/cm²:1.6%

	5.6 µg/cm²: 12.5% 55.6 µg/cm²: 42.2% 277.8 µg/cm²: 65.6% 1388.9 µg/cm²: 87.5%	0.4 µg/cm²: 3.1% 4.4 µg/cm²: 29.7% 22.2 µg/cm²: 57.8% 111.1 µg/cm²: 82.8%
Controls	No reactions to vehicle in the patients included into analysis	
Other information	See figure below. Three patients were excluded from the study, so results are based on 64 patients.	

Figure 4.3 – 1: Dose-response curve of 64 patients sensitised to HICC, according to a previous PT, regarding two preparations: perfume and cream, the rhomboid and dot symbol, respectively, indicating the observed response. The curve was fitted by a logistic function (10).



Isoeugenol

Isoeugenol *(11)*	
Design	blinded, randomised doses and controlled
Test subjects	20 patients with a positive patch test to isoeugenol
Controls	20 healthy controls
Substance	purity: 98%
Patch test	20 mg solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2% to 0.01% (8 steps)
-control/vehicle	petrolatum
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line
ROAT	outer aspect of upper arms
area	5 x 5 cm (2 areas: one test and one control)
applications/day	two with roll-on
dose	0.2% in ethanol
µg /application/cm^2	Doses measured to 0.14 -0.13 mg/application the first 14 days = 5.6 µg/cm^2
control substance	ethanol
definition of positive	any degree of reaction
period	Two weeks at upper arm and if negative another two weeks including application to base of neck
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	0.08% 32 µg/cm^2
PT no effect level (observed)	< 0.01% = 0.4 µg/cm^2
ROAT	
Dose: 0.2%	12/19 (63%)
Controls	No reactions were seen
Other information	

Isoeugenol *(12)*	
Design	blinded, randomised
Test subjects	27 patients with a positive patch test to isoeugenol
Controls	20 healthy controls
Substance	purity: 98%
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2% to 0.00006% (17 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of reactions to higher concentrations
ROAT	volar aspect of lower arm
area	3 x 3 cm (2 areas)
applications/day	two with droplet bottle (30 mg per application)
dose	0.05% in ethanol and 0.2%
µg /application/cm^2	Doses were calculated as mean 2.2 µg/cm^2 (low conc.) and 9 µg/cm^2 (high conc.)
control substance	ethanol
definition of positive	clear visible erythema
period	28 days
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	/
PT no effect level (observed)	< 0.0005% (5 ppm)
ROAT	Cumulative responses
Dose 1: 0.05%	10/24 (42%)
Dose 2: 0.2%	16/24 (67%)
Controls	No reactions were seen
Other information	Response to the low concentration in the ROAT appeared after median 15 days and to the high concentration after median 7 days.

Isoeugenol *(13)*	
Design	blinded, randomised and controlled
Test subjects	13 patients with a positive patch test to isoeugenol and 4 in part 1 (pre-test)
Controls	10 healthy controls (dermatitis patients)
Substance	purity: /
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2% to 0.00006% (w/v) (16 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration elicitating at least + reaction
ROAT	Axilla
area	10 x 10 cm^2 (estimated)
applications/day	two with roll-on deodorant (117-586 mg per application of solution) average cases: 266 mg/application controls: only range given
dose	Part 1: Step 1:0.02% Step 2: 0.063% Step 3:0.2% Part 2: Step1:0.0063% Step 2:0.02% Step 3: 0.063%
dose/application/cm^2	Part 2: Step 1: 0.167 Step 2: 0.53 Step 3: 1.67 µg/application/ cm^2 (calculated based on data)
control substance	deodorant matrix
definition of positive	eczematous response covering 25% of test area
period	Part one: one week with each concentration: maximum three weeks Part two: two weeks with each concentration: maximum six weeks
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	/
PT no effect level (observed)	<0.0005% (0.15 µg/cm^2)
ROAT	
Step 1 (0.0063%)	3/13 (23%)
Step 2 (0.02%)	9/13 (69%)
Step 3 (0.063%)	10/13 (77%)
Controls	No reactions were seen
Other information	Deodorants containing cinnamal were responsible for all reactions in cinnamal sensitized individuals (p<0.001) and all control persons were negative (p<0.001)

References

1 Johansen J D, Andersen K E, Svedman C, Bruze M, Bernard G, Gimenez-Arnau E, Rastogi S C, Lepoittevin J P, Menne T. Chloroatranol, an extremely potent allergen hidden in perfumes: a dose-response elicitation study. *Contact Dermatitis* 2003: **49**: 180-4.

2 Johansen J D, Bernard G, Gimenez-Arnau E, Lepoittevin J P, Bruze M, Andersen K E. Comparison of elicitation potential of chloroatranol and atranol--2 allergens in oak moss absolute. *Contact Dermatitis* 2006: **54**: 192-5.

3 Johansen J D, Andersen K E, Rastogi S C, Menne T. Threshold responses in cinnamic-aldehyde-sensitive subjects: results and methodological aspects. *Contact Dermatitis* 1996: **34**: 165-71.

4 Bruze M, Johansen J D, Andersen K E, Frosch P, Lepoittevin J P, Rastogi S, Wakelin S, White I, Menne T. Deodorants: an experimental provocation study with cinnamic aldehyde. *J Am Acad Dermatol* 2003: **48**: 194-200.

5 Svedman C, Bruze M, Johansen J D, Andersen K E, Goossens A, Frosch P J, Lepoittevin J P, Rastogi S, White I R, Menne T. Deodorants: an experimental provocation study with hydroxycitronellal. *Contact Dermatitis* 2003: **48**: 217-23.

6 Heydorn S, Menne T, Andersen K E, Bruze M, Svedman C, Basketter D, Johansen J D. The fragrance hand immersion study - an experimental model simulating real-life exposure for allergic contact dermatitis on the hands. *Contact Dermatitis* 2003: **48**: 324-30.

7 Johansen J D, Frosch P J, Svedman C, Andersen K E, Bruze M, Pirker C, Menne T. Hydroxyisohexyl 3-cyclohexene carboxaldehyde- known as Lyral: quantitative aspects and risk assessment of an important fragrance allergen. *Contact Dermatitis* 2003: **48**: 310-6.

8 Jorgensen P H, Jensen C D, Rastogi S, Andersen K E, Johansen J D. Experimental elicitation with hydroxyisohexyl-3-cyclohexene carboxaldehyde-containing deodorants. *Contact Dermatitis* 2007: **56**: 146-50.

9 Fischer L A, Menné T, Avnstorp C, Kasting G B, Johansen J D. Hydroxyisohexyl 3-cyclohexene carboxaldehyde allergy: relationship between patch test and repeated open application test thresholds. *Br J Dermatol* 2009: **161**: 560-7.

10 Schnuch A, Uter W, Dickel H, Szliska C, Schliemann S, Eben R, Rueff F, Gimenez-Arnau A, Loffler H, Aberer W, Frambach Y, Worm M, Niebuhr M, Hillen U, Martin V, Jappe U, Frosch P J, Mahler V. Quantitative patch and repeated open application testing in hydroxyisohexyl 3-cyclohexene carboxaldehyde sensitive-patients. *Contact Dermatitis* 2009: **61**: 152-62.

11 Johansen J D, Andersen K E, Menné T. Quantitative aspects of isoeugenol contact allergy assessed by use and patch tests. *Contact Dermatitis* 1996: **34**: 414-8.

12 Andersen K E, Johansen J D, Bruze M, Frosch P J, Goossens A, Lepoittevin J P, Rastogi S, White I, Menne T. The time-dose-response relationship for elicitation of contact dermatitis in isoeugenol allergic individuals. *Toxicol Appl Pharmacol* 2001: **170**: 166-71.

13 Bruze M, Johansen J D, Andersen K E, Frosch P, Goossens A, Lepoittevin J P, Rastogi S C, White I, Menne T. Deodorants: an experimental provocation study with isoeugenol. *Contact Dermatitis* 2005: **52**: 260-7.

Inhalation Toxicology, 18:685–706, 2006

ISSN: 0895-8378 print / 1091-7691 online
DOI: 10.1080/08958370600742680

Taylor & Francis
Taylor & Francis Group

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

Roger A. Renne
Battelle, Toxicology Northwest, Richland, Washington, USA

Hiroyuki Yoshimura
Japan Tobacco, Inc., Tokyo, Japan

Kei Yoshino
Japan Tobacco, Inc., Kanagawa, Japan

George Lulham
JTI Macdonald Corp., Toronto, Canada

Susumu Minamisawa
Japan Tobacco, Inc., Tokyo, Japan

Albrecht Tribukait
Japan Tobacco, Inc., Germany, Cologne, Germany

Dennis D. Dietz, Kyeonghee Monica Lee, and R. Bruce Westerberg
Battelle, Toxicology Northwest, Richland, Washington, USA

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.

Received 2 January 2006; accepted 31 March 2006.
The authors are grateful to the following staff for their valuable contributions to this work: J. C. Blessing, M. L. Clark, K. M. Gideon, B. K. Hayden, J. D. Penner, J. T. Pierce, B. L. Thomas, and R. L. Thomas.
Address correspondence to Roger Renne, PO Box 999, Richland, WA 99352, USA. E-mail: renne@battelle.org

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

	Percent of blend component in cigarettes	
Blend components	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 $22 \pm 1°C$ 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 *para*-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	*trans*-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*H*)-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-Butylidenphthalide	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(2*H*)-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.
[a]Chemical Abstract Service registry number.
[b]The Flavor and Extract Manufacturers Association reference number.
[c]Code of Federal Regulations reference to Title 21 indicating regulatory status of material.
[d]Council 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.
[a]Chemical Abstract Service registry number.
[b]The Flavor and Extract Manufacturer's Association reference number.
[c]Code of Federal Regulations reference to Title 21 indicating regulatory status of material.
[d]Council 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 ±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 (NEWCAS; 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 (Crl: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 moribundity. 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 (CO_2)-anesthetized animals into tubes containing potassium ethylenediaminete traacetic 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% CO_2 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 supravitally with new methylene blue and enumerated as reticulocytes per 1000 enthrocytes 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% CO_2 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 μm. 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 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.

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

Parameter	Target	Run average: Test cigarette	Run average: 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.

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.

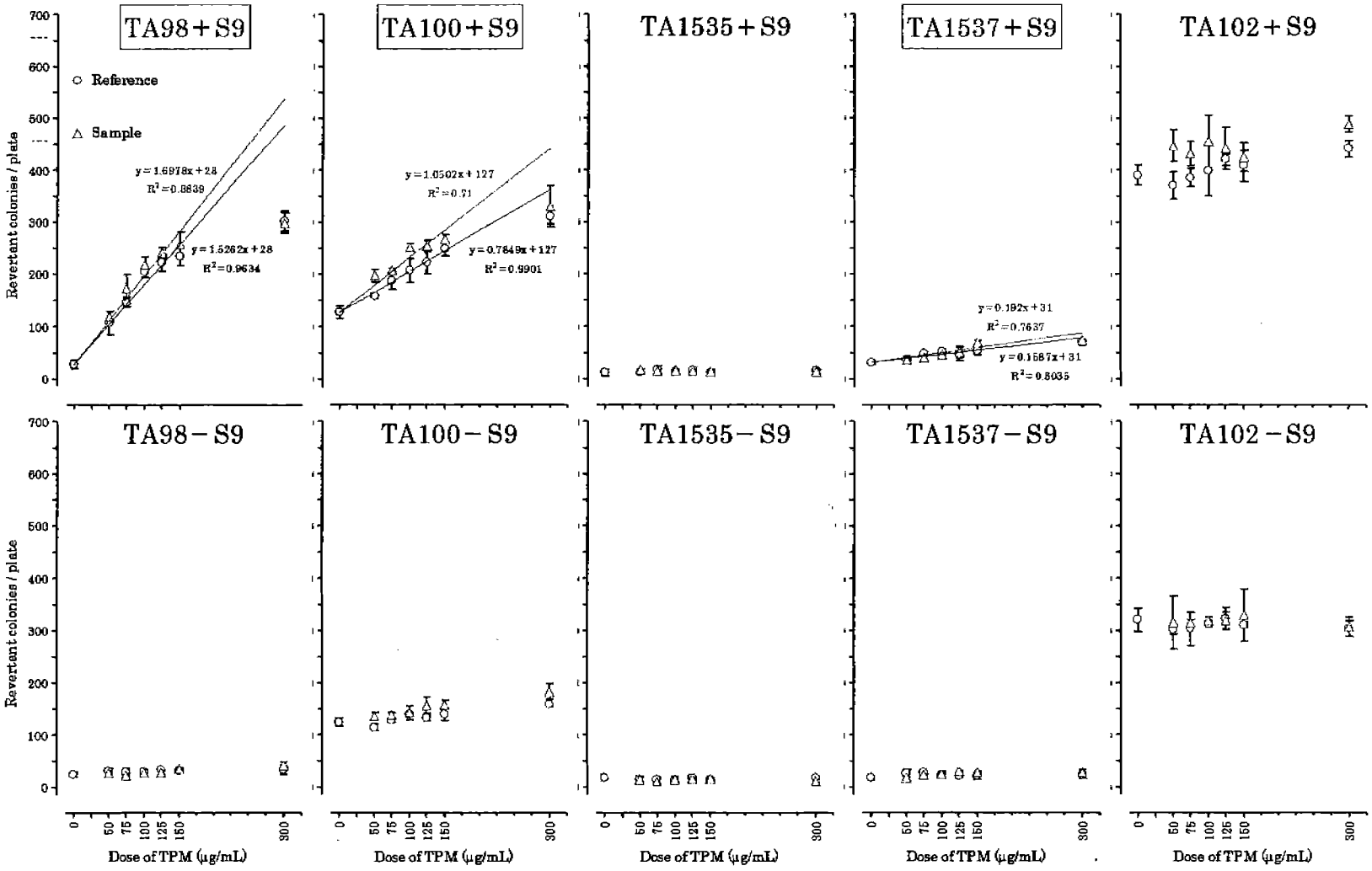
Body Weights and Clinical Observations

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.

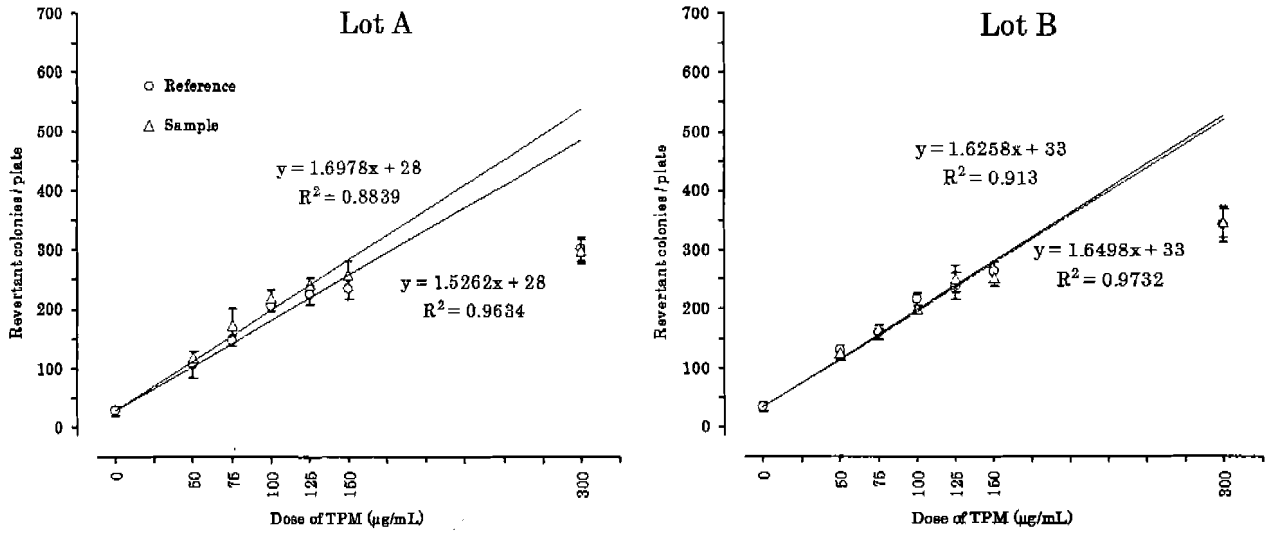
Organ Weights

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. 1. Ames assay results, study 1 cigarettes.



MEAN ± SD of Specific Activity (50 to 150 µg/plate)

Reference	1576 ± 141.9	Reference.....	1734 ± 170.9
Sample.....	1783 ± 167.3	Sample.....	1703 ± 151.2

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

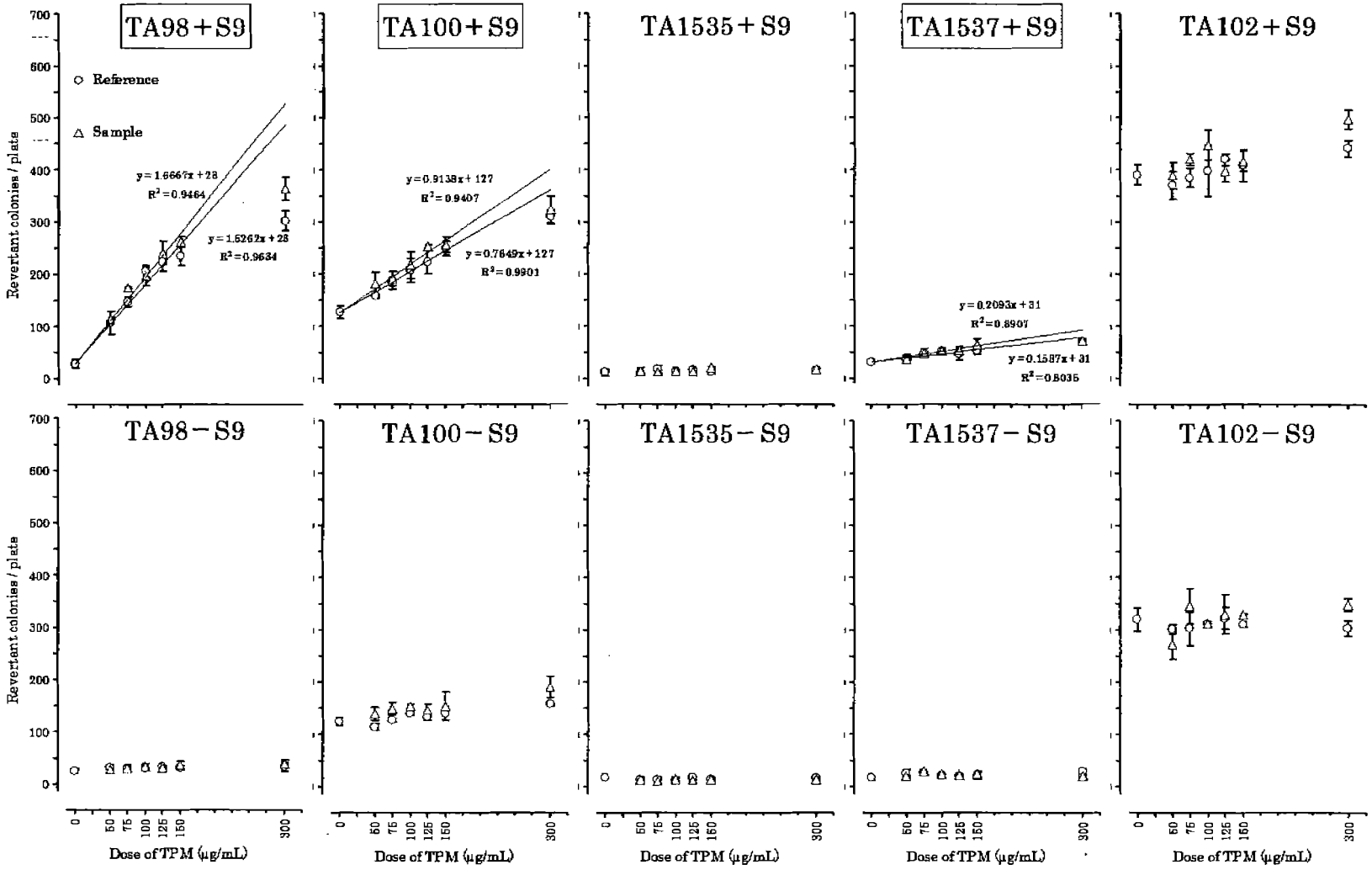
There were occasional statistically significant differences in hematology and clinical chemistry parameters from control values in groups exposed to smoke from test or reference cigarettes in both study 1 and study 2. These differences did not occur in a dose-response pattern and were well within ±2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



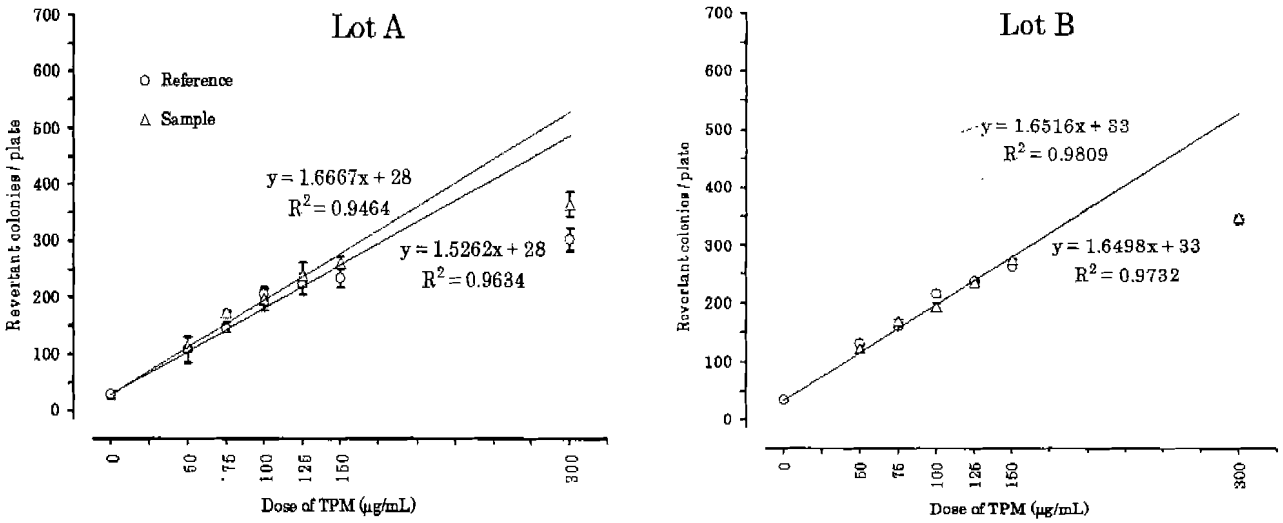
N=2. Only the first lot (Lot A) is indicated in this figure.
The second lot (Lot B) showed the same tendency as the first lot .

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

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



MEAN±SD of Specific Activity (50 to 150 µg/plate)

Reference.....	1576±141.9	Reference.....	1734±170.9
Sample.....	1726±138.6	Sample-1.....	1701±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 ± SD (%CV)]				
	Measured exposure concentration (mg WTPM/L; $n = 134$)	Nicotine concentration (μg/L; $n = 28$)	CO concentration (ppm; $n = 67$)	Percent of target WTPM concentration (mean ± SD)	Particle size (MMAD, μm)
Test target exposure concentration (mg WTPM/L)					
0.8	0.798 ± 0.040 (5.0)	56.8 ± 2.6 (4.6)	646 ± 34 (5.3)	100 ± 5	0.65 ± 0.01
0.2	0.194 ± 0.007 (3.6)	12.9 ± 0.6 (4.7)	158 ± 9 (5.7)	97 ± 4	0.62 ± 0.04
0.060	0.060 ± 0.002 (3.3)	4.0 ± 0.2 (5.0)	54 ± 3 (5.6)	100 ± 3	0.66 ± 0.03
Reference target exposure concentration (mg WTPM/L)					
0.8	0.784 ± 0.031 (4.0)	55.1 ± 2.3 (4.2)	676 ± 31 (4.6)	98 ± 4	0.57 ± 0.03
0.2	0.201 ± 0.004 (1.8)	13.0 ± 0.4 (3.4)	170 ± 15 (8.7)	100 ± 2	0.64 ± 0.07
0.060	0.060 ± 0.002 (3.3)	4.1 ± 0.2 (4.4)	57 ± 3 (5.8)	99 ± 3	0.66 ± 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

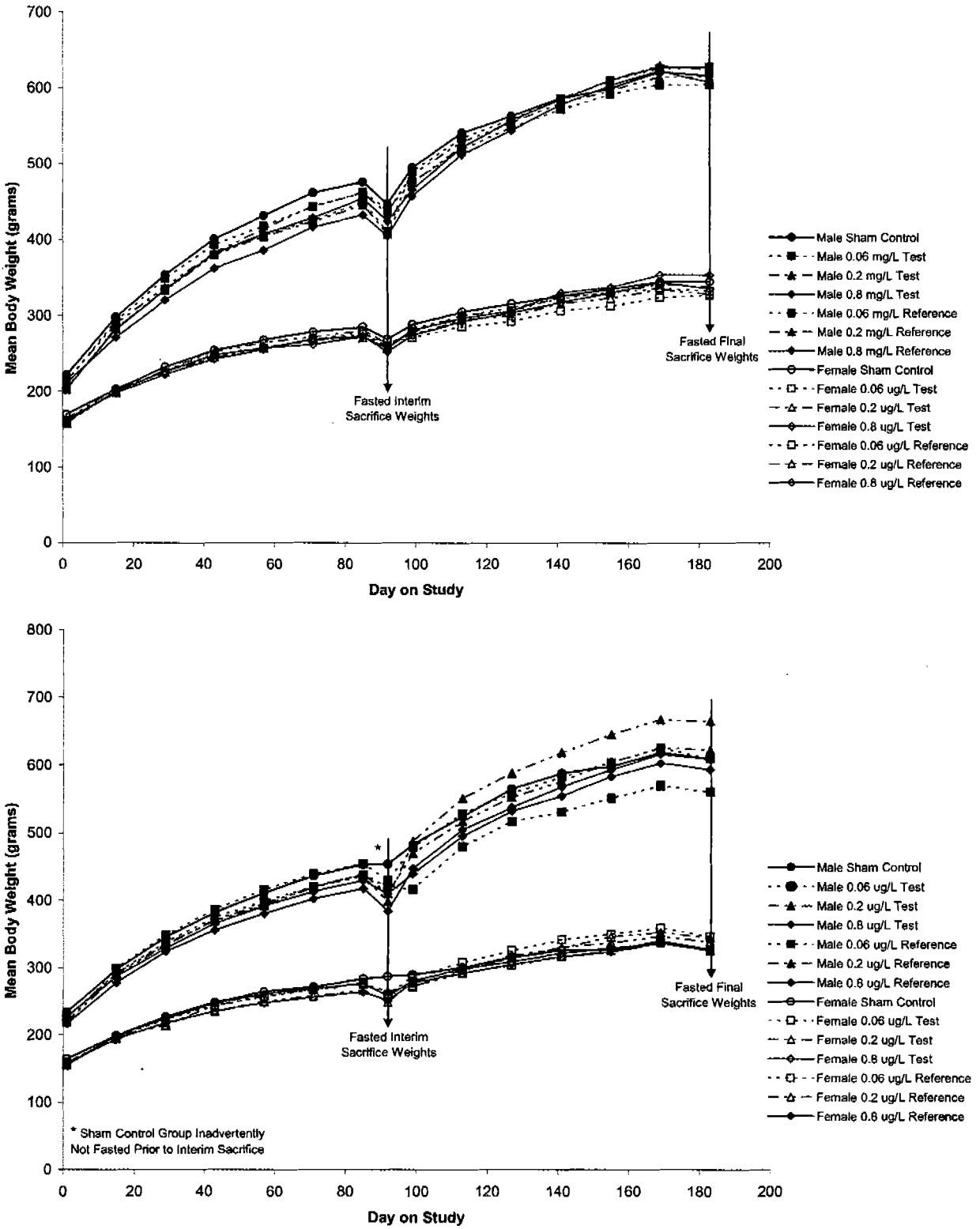


FIG. 5. Body weights, study 1 (top) and study 2 (bottom).

TABLE 8
Organ weights for rats exposed to smoke from study 1 cigarettes ($n = 20$, g ± 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	153 ± 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

Organ/diagnosis	Incidence of lesions (mean severity, if applicable) by target exposure concentration (mg WTPM/L)						
		Test			Reference		
	Sham controls	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.
[a]Number of tissues or animals examined.
[b]Number 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

Organ/diagnosis	Incidence of lesions (mean severity, if applicable) by target exposure concentration (mg WTPM/L)						
		Test			Reference		
	Sham controls	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.
[a]Number of tissues or animals examined.
[b]Number 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.

REFERENCES

Ayres, P., Mosberg, A. T., and Coggins, C. R. 1990. Modernization of nose-only smoking machines for use in animal studies. *J. Am. Coll. Toxicol.* 9:441–446.

Ayres, P. H., Hayes, J. R., Higuchi, M. A., Mosberg, A. T., and Sagartz, J. W. 2001. Subchronic inhalation by rats of mainstream smoke from a cigarette that primarily heats tobacco compared to a cigarette that burns tobacco. *Inhal. Toxicol.* 13:149–186.

Baker, R. R., and Bishop, L. J. 2004. The pyrolysis of tobacco ingredients. *J. Anal. Appl. Pyrol.* 71:223–311.

Baker, R. R., Massey, E. H., and Smith, G. 2004. An overview of the effects of tobacco ingredients on smoke chemistry and toxicity. *Food Chem. Toxicol.* 42:S53–S83.

Baumgartner, H., and Coggins, C. R. E. 1980. Description of a continuous-smoking inhalation machine for exposing small animals to tobacco smoke. *Beitr. Tabakforsch. Int.* 10:169–174.

Brecher, G., and Schneiderman, M. 1950. A time-saving device for the counting of reticulocytes. *Am. J. Clin. Pathol.* 20:1079.

Burger, G. T., Renne, R. A., Sagartz, J. W., Ayres, P. H., Coggins, C. R. E., Mosberg, A. T., and Hayes, A. W. 1989. Histologic changes in the respiratory tract induced by inhalation of xenobiotics: Physiologic adaptation or toxicity? *Toxicol. Appl. Pharmacol.* 101:521–542.

Carmines, E. L. 2002. Evaluation of the potential effects of ingredients added to cigarettes. Part 1: Cigarette design, testing approach, and review of results. *Food Chem. Toxicol.* 40:77–91.

Coggins, C. R. E., Fouillet, X. L., Lam, R., and Morgan, K. T. 1980. Cigarette smoke induced pathology of the rat respiratory tract. A comparison of the effects of the particulate and vapor phases. *Toxicology* 16:83–101.

Coggins, C. R. E., Duchosal, F., Musy, C., and Ventrone, R. 1981. The measurement of respiratory patterns in rodents, using whole body plethysmography and pneumotachography. *Lab. Anim.* 15:137–140.

Coggins, C. R. E., Ayres, P. H., Mosberg, A. T., and Burger, G. T. 1989. Comparative inhalation study in rats, using a second prototype of a cigarette that heats rather than burns tobacco. *Inhal. Toxicol.* 1:197–226.

Dalbey, W. E., Nettesheim, P., Griesemer, R., Caton, J. E., and Guerin, M. R. 1980. Chronic inhalation of cigarette smoke by F344 rats. *J. NCI.* 64:383–390.

Gaworski, C. L., Dozier, M. M., Gerhart, J. M., Rajendran, N., Brennecke, L. H., Aranyi, C., and Heck, J. D. 1997. 13-wk inhalation study of menthol cigarette smoke. *Food Chem. Toxicol.* 35:683–692.

Gaworski, C. L., Dozier, M. M., Heck, J. D., Gerhart, J. M., Rajendran, N., David, R. M., Brennecke, L. H., and Morrisey, R. 1998. Toxicologic evaluation of flavor ingredients added to cigarette tobacco: 13-wk inhalation exposures in rats. *Inhal. Toxicol.* 10:357–381.

Gopinath, C., Prentice, D. E., and Lewis, D. J. 1987. *Atlas of experimental toxicologic pathology*. Lancaster, PA: MTP Press.

Hill, M. A., Watson, C. R., and Moss, O. R. 1977. *NEWCAS—An interactive computer program for particle size analysis*. PNL-2405. Richland, WA: Battelle Pacific Northwest Laboratories.

Hoffman, D., and Hoffman, I. 1997. The changing cigarette, 1950–1995. *J. Toxicol. Environ. Health* 50:307–364.

Hoffman, D., and Hoffman, I. 2001. The changing cigarette: chemical studies and bioassays. In *National Cancer Institute (NCI) Monograph 13, Risks associated with smoking cigarettes with low machine-measured yields of tar and nicotine*, pp. 159–191. U.S. Department of Health and Human Services, Public Health Service, National Institute of Health, National Cancer Institute, Bethesda, MD, USA.

LaVoie, E. J., Hecht, S. S., Hoffman, D., and Wynder, E. L. 1980. The less harmful cigarettes and tobacco smoke flavours. In *Banbury Report 3, A Safe Cigarette?* eds. G. B. Gori and F. G. Back, pp. 251–260. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Lewis, D. J. 1980. Factors affecting the distribution of tobacco smoke-induced lesions in rodent larynx. *Toxicol. Lett.* 9:189–194.

Lewis, D. J. 1991. Morphologic assessment of pathological changes within the rat larynx. *Toxicol. Pathol*, 19:352–357.

National Academy of Sciences. 1996. *Guide for the care and use of laboratory animals*. Washington, DC: Institute of Laboratory Animal Resources, Commission on Life Sciences, National Reserch Council. National Academy Press.

Paschke, T., Scherer, G., and Heller, W. F. 2002. Effects of ingredients on cigarette smoke composition and biological activity: A literature review. *Beitr. Tabakforsch. Int./Contrib. Tobacco Res.* 20:107–247.

Renne, R. A., Gideon, K. M., Miller, R. A., Mellick, P. W., and Grumbein, S. L. 1992. Histologic methods and interspecies variations in the laryngeal histology of F344/N rats and B6C3F1 mice. *Toxicol. Pathol.* 20:44–51.

Rodgman, A. 2002a. Some studies of the effects of additives on cigarette mainstream smoke properties. I. Flavorants. *Beitr. Tabakforsch. Int.* 20:83–103.

Rodgman, A. 2002b. Some studies of the effects of additives on cigarette mainstream smoke properties. II. Casing materials. *Beitr. Tabakforsch. Int.* 20:279–299.

Rodgman, A., and Green, C. R. 2002. Toxic chemicals in cigarette mainstream smoke—Hazard and hoopla. *Beitr. Tabakforsch. Int.* 20:481–545.

Roemer, E., Tewes, F. J., Mesigen, T. J., Veltel, D. J., and Carmines, E. L. 2002. Evaluation of the potential effects of ingredients added to cigarettes. Part 3: *In vitro* genotoxicity and cytotoxicity. *Food Chem. Toxicol.* 40:105–111.

Rustemeier, K., Stabbert, R., Haussmann, H. J., Roemer, E., and Carmines, E. L. 2002. Evaluation of the potential effects of ingredients added to cigarettes. Part 2: Chemical composition of mainstream smoke. *Food Chem. Toxicol.* 40:93–104.

Siegel, S. 1956. *Non-parametric statistics for the behavioral sciences*. New York: McGraw-Hill.

Vanscheeuwijck, P. M., Teredesai, A., Terpstra, P. M., Verbeeck, J., Kuhl, P., Gerstenberg, B., Gebel, S., and Carmines, E. L. 2002. Evaluation of the potential effects of ingredients added to cigarettes. Part 4: Subchronic inhalation toxicity. *Food Chem. Toxicol.* 40:113–131.

Wehner, A. P., Renne, R. A., Greenspan, B. J., DeFord, H. S., Ragan, H. A., Westerberg, R. B., Wright, C. W., Buschbom, R. L., Burger, G. T., Hayes, A. W., Coggins, C. R. E., and Mosberg, A. T. 1990. Comparative subchronic inhalation bioassay in hamsters of a cigarette that only heats tobacco. *Inhal. Toxicol.* 2:255–284.

World Health Organization. 2001. *Advancing knowledge on regulating tobacco products*, pp. 40–46. Geneva: WHO.

Wynder, E. L., and Hoffman, D. 1967. *Tobacco and tobacco smoke. Studies in experimental carcinogenesis*, pp. 526–528. New York: Academic Press.

Young, J. T. 1981. Histopathologic examination of the rat nasal cavity. *Fundam. Appl. Toxicol.* 1:309–312.

European Medicines Agency
Evaluation of Medicines for Human Use

London, 4 September 2008
Doc. Ref. EMEA/HMPC/349465/2006

ASSESSMENT REPORT ON
MENTHA X PIPERITA L., AETHEROLEUM

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Tel. (44-20) 74 18 84 00 Fax (44-20) 75 23 70 51
E-mail: mail@emea.eu.int http://www.emea.eu.int

I. REGULATORY STATUS OVERVIEW

MA: Marketing Authorisation;
TRAD: Traditional Use Registration;
Other TRAD: Other national Traditional systems of registration;
Other: If known, it should be specified or otherwise add 'Not Known'

Member State	Regulatory Status			
Austria	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:
Belgium	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:
Cyprus	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:
Czech Republic	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:
Denmark	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:
Estonia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:
Finland	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:
France	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:
Germany	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:
Greece	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:
Hungary	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:
Iceland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:
Ireland	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:
Italy	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify: Food supplements
Latvia	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify: Natural products
Liechtenstein	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:
Lithuania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:
Luxemburg	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:
Malta	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:
The Netherlands	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:
Norway	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:
Poland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:
Portugal	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:
Slovak Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:
Slovenia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:
Spain	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:
Sweden	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:
United Kingdom	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:

II. ASSESSMENT REPORT FOR HERBAL SUBSTANCE(S), HERBAL PREPARATION(S) OR COMBINATIONS THEREOF WITH WELL-ESTABLISHED USE

Mentha x piperita L., aetheroleum

BASED ON ARTICLE 10A OF DIRECTIVE 2001/83/EC AS AMENDED

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Mentha x piperita* L., aetheroleum
Herbal preparation(s)	Menthae piperitae aetheroleum
Pharmaceutical forms	Liquid dosage forms
Rapporteur	Dr Helena Pinto Ferreira Dr Ana Paula Martins

1 INTRODUCTION

Peppermint is believed to be a hybrid of spearmint (*Mentha spicata* L.*)* and water mint (*Mentha aquatica* L.*)* (Murray, Lincoln and Marble, 1972).
It has been a popular domestic remedy for at least two centuries. The essential oil is obtained from the fresh leaves of *Mentha piperita L.* by steam distillation and its most active product available in most parts of the world for flavouring, cosmetic and medicinal uses.

The English Dictionary of Medicinal and Surgical Knowledge, in 1800, already considered peppermint oil as "an aromatic stimulant to allay nausea, relieve spasmodic pain to the stomach and the bowels, expel flatus or cover the taste or the quality of gripping effects of other medicine"

The activity of peppermint oil and of its major constituent, menthol, have been subject to a series of pharmacological and clinical studies. Several medicinal products have been authorized for the relief of digestive disorders, to reduce spasms of the smooth muscles, for neuralgic pains and for colds and coughs, given orally or topically.

This monograph gives the result of the literature available on the efficacy and safety of peppermint oil, for well-established use.

1.1 Description of the herbal substance(s), herbal preparation(s) or combinations thereof

- Herbal substance(s)[1] [2]:

Mentha x piperita L., aetheroleum

- Herbal preparation(s)[1] [2]:

Menthae piperitae aetheroleum

- Combinations of herbal substance(s) and/or herbal preparation(s)[3]

1.2 Information on period of medicinal use in the Community regarding the specified indication

2 NON-CLINICAL DATA

For all studies cited, it should be stated by means of a detailed description which herbal substance(s)/herbal preparation(s) have been used and information should be provided for each preparation separately.

2.1 Pharmacology

2.1.1 Overview of available data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

[1] According to "Note for guidance on Quality of herbal medicinal products" (CPMP/QWP/2819/00…)
[2] According to "Note for guidance on Specifications: Test procedures and acceptance criteria for herbal drugs, herbal preparations and herbal medicinal products" (CHMP/QWP/2820/00)
[3] According to the Guideline on the clinical assessment of fixed combinations of herbal substances/herbal preparations (EMEA/HMPC/166326/2005)

Major chemical constituents

The major constituents are menthol (30-55%) and menthone (14-32%). Other monoterpenes present are limonene (1-5%), cineole (3, 5-14%), menthofuran (1-9%), isomenthone (1,5-10%), menthyl acetate (2,8-10%), pulegone (until 4%), carvone (until 1%) with a ratio of cineole content to limonene content greater than 2.

Antispasmodic action on the smooth muscle

Peppermint oil as a 1 % emulsion exhibited relaxant effects on tracheal smooth muscle of the guinea pig: the I_{50} was 83-91 mg/L.

Peppermint oil emulsified with tween, 1% in aqueous solution, relaxed chemically contracted guinea pig taenia coli (I_{50}: 22.1 μg/mL) and inhibited spontaneous activity in the guinea pig colon (I_{50}: 25.9 μg/mL) and rabbit jejunum (I_{50}: 15.2 μg/mL). Using whole cell clamp configuration in these jejunal muscle cells, the potential –dependent calcium currents were inhibited in a dose-dependent manner by peppermint oil. Peppermint oil reduced the peak current amplitude and increased the rate of current decay, indicating a reduction of calcium influx similar to that caused by dihydropyridine calcium antagonists. Peppermint oil demonstrated to inhibit non-competitively 5 –hidroxitriptamine (serotonin) and the substance P induced smooth muscle contraction (Hills JM et al, 1991).

Both menthol and peppermint oil inhibited specific [3H] nitrendipine and [3H] PN 200-110 binding to smooth and cardiac muscle and neuronal preparations with potencies comparable to, but slightly lower than, those measured in the pharmacological and 45Ca2+ uptake experiments. Binding of menthol and peppermint oil, studied at 78 micrograms ml-1, was competitive against [3H] nitrendipine in both smooth muscle and synaptosome preparations. The data indicate that both menthol and peppermint oil exert Ca2+ channel blocking properties which may underlie their use in irritable bowel syndrome. The authors conclude that Ca2+ channel antagonism may not be the only pharmacological effect of menthol and peppermint oil contributing to intestinal smooth muscle relaxation (Hawthorn M et al, 1988).

Another study made experiments on male guinea pigs concerning the pharmacological activity of essential oils on Oddi`s sphincter. Oddi`s sphincter prolapses through i.v. injection of *Mentha piperita* L. **(**Anon, 1990).

Peppermint oil appears to enhance production of bile. In experiments where bile flowed out of a cannula from an anaesthetized dog, an infusion of peppermint leaves (0.4 g/kg) enhanced bile production. Menthol also produced an enhancement of bile production: 0.06 g/kg in 1 dog and 0.1-1.0 g/kg in rats.

In others experimental studies in animals, menthol and peppermint oil induced a marked and dose related choleresis (Siegers C., Guo Z., Pentz R, 1991).

Ant carminative activity

Peppermint oil showed antifoaming and carminative activity in vitro. Reductions in gastric and intestinal foam volume were observed in vitro studies with peppermint oil. The carminative effect results from a combination of actions. Antifoaming activity associated to the relaxation of the oesophageal sphincter may release the gastric gas. The antimicrobial activity helps to reduce the intestinal gas (Harries N., James K., Pugh W, 1978)

Analgesic action

To characterize the effects of peppermint and caraway oil individually and in combination on the visceral nociception in a rat model of post-inflammatory hyperalgesia, a study was performed. 28 male Lewis rats were randomized to treatment with a rectal administration of trinitrobenzene sulphonic acid (TNBS)/ethanol or physiological saline solution. After 14 days of treatment with peppermint and/or caraway oil, a reduced visceromotor response was found of up to 50 % compared to placebo.

Individually both oils had no significantly effect on post-inflammatory visceral hyperalgesia (Adam B et al, 2006).

Studies have demonstrated that rodents who lay down in bedding that was soaked in peppermint oil show a pain relief response compared with those who lay in control bedding.

On another study in identified Helix neurons, the authors indicate a modulating action of external menthol on Ca inactivation (Hawthorn M et al, 1988)

Virucidal, antimicrobial and antiplasmid action

The virucidal effect *in vitro* was assessed on a study, where the inhibitory activity against herpes simplex (type 1 and type 2) was tested. A plaque reduction assay was used with RC-37 cells, where the HSV-1 and 2 were grown. Peppermint oil was dissolved in ethanol (1% final concentration of ethanol) and added to the cell culture medium, at the non-toxic concentration of 0, 01%. To determine the antiviral action, cells were pre-treated with peppermint oil before the infection, viruses were incubated with peppermint oil before infection and cells and viruses were incubated together during adsorption or after penetration of the virus into the host cells. All these experiments were performed in parallel with acyclovir to test the suitability of the assay and were compared to untreated controls. Ethanol had no effect on virus titters and did not exhibit any toxic effect on the cells. At non-cytotoxic concentration of the oil, 0, 01% peppermint oil, the titres of HSV-1 and 2 reduced 82% and 92% respectively. Higher concentrations reduced virus titters for more than 90 %. The 50% inhibitory concentration (IC_{50}) of peppermint oil was determined at 0,002% and 0, 0008% for HSV-1 and 2. The peppermint oil affected the virus before adsorption, exerting a direct effect on the virus. Not after penetration into the host cell (Dresser et al, 2002).

Peppermint oil showed antimicrobial and antiplasmid activity, demonstrating a synergistic additive interaction with oxytetracycline (Schelz Z, 2006).

Bronchomucotropic activity

Menthol

Menthol (1mg of menthol/kg added to the water vaporizer, corresponding to systemic absorption of not over 20 µg/kg body weight) was given to rabbits anesthetised with urethane. It augmented the soluble mucus content and lowered the specific gravity of respiratory tract fluid. The author concludes that the bronchomucotropic effects were due to direct local stimulation of mucus secreting cells in the respiratory tract. Inhalation of larger amounts of menthol depressed the volume output and mucus content of respiratory tract fluid (Boyd., Sheppard , 1969).

On several old studies peppermint oil was reported to depress ciliary activity, but there are some other studies where PO markedly stimulated it (Das, Rathor, Sinha, Santal, 1970) .

Using VapoRub vapours in a study, where animals were exposed continuously to 30 times the relative peak clinical atmospheric concentrations of the product, no significant suppression of pulmonary bactericidal activity was observed (Jakab, Green, 1975).

Interactions

Peppermint oil has demonstrated competitive antagonism at calcium channels in animals and in vitro. On a theoretical point of view, the calcium channels blockers effectivity may be modified.

Peppermint oil was reported to inhibit cytochrome P450 3A (CYP3A) activity in rat and human liver microsomes and to enhance the oral bioavailability of the CYP3A4 substrate felodipine in people (Dresser et al, 2002).

A study compared the effects of peppermint oil with ketoconazole and D-alpha-tocopheryl poly (ethylene glycol 1000) succinate (TPGS), on the inhibition of cyclosporine oral bioavailability in rats. Peppermint oil (100mg/kg) tripled the mean cyclosporine maximum concentration. The author

suggests that inhibition of cytochrome P450 3A is not the only mean by which peppermint oil enhances cyclosporine bioavailability (Wacher et al, 2002).

Peppermint oil demonstrated to enhance 46-fold increase the penetration of 5-fluorouracil, in a study using excised rat skin (Abdullah et al 1996**)**.

2.1.2 Assessor's overall conclusions on pharmacology

Peppermint showed in vitro and in vivo studies, to have antispasmodic activity on the gastrointestinal smooth muscle. The mechanism seems to be related to the reduction of the calcium influx and the block of non-competitive contraction induced by 5-hidroxytriptamine.

Peppermint appears to have antiseptic properties in vitro and cholagogic action in vivo, but had no significantly effect on post-inflammatory visceral hyperalgesia.

The bronchomucotropic effects were contradictory, with depressing and stimulatory action of mucus secreting cells in the respiratory tract.

The competitive antagonism at calcium channels in animals and in vitro raises the possibility of interaction with other calcium blockers.

The reversible inhibition of cytochrome P450 3A was reported in vitro and in vivo, requiring further investigation.

Cyclosporine maximum concentration may increase, with the action of peppermint oil. Topically, peppermint oil increased the penetration of 5-fluorouracil.

2.2 Pharmacokinetics

2.2.1 Overview of available data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

Dermal absorption

The absorption rate for Peppermint oil was measured after the application of eserine in a peppermint oil vehicle, to a 2.2cm^2 shaved area on the abdomen of mice. The latent period between application and the eserine-induced signs, gave the absorption rate of peppermint oil, which was of 58 minutes (Final report on the Safety Assessment of Mentha Piperita, 2001)

Inhalation

Pulmonary absorption depends on various factors, like the kind of compound and the breathing mechanics of the subjects. In one study, it was demonstrated that the release of compounds from water into the headspace depended on water temperature.

Elimination half lives for inhalated menthol and camphor were 35, 5 and 39,9min respectively. This indicates that there should be no accumulation during long-term application (Kohlert et al, 2000).

Oral absorption and metabolism

The major biliary metabolite is menthol glucuronide, which undergoes enterohepatic circulation.

Metabolism of l-menthol in rats was investigated both *in vivo* and *in vitro*. Metabolites isolated and characterized from the urine of rats after oral administration (800 mg/kg of body weight/day) of l-menthol were the following: p-menthane-3, 8-diol (II), p-menthane-3, 9-diol (III), 3, 8-oxy-p-menthane-7-carboxylic acid (IV), and 3, 8-dihyroxy-p-menthane-7-carboxylic acid (V). *In vivo*, the major urinary metabolites were compounds II and V. Repeated oral administration (800 mg/kg of body weight/day) of l-menthol to rats for 3 days resulted in the increase of both liver microsomal

cytochrome P-450 content and NADPH-cytochrome c reductase activity by nearly 80%. Further treatment (for 7 days total) reduced their levels considerably, although the levels were still higher than the control values. Both cytochrome b5 and NADH-cytochrome c reductase levels were not changed during the 7 days of treatment. Rat liver microsomes readily converted l-menthol to p-menthane-3, 8-diol (II) in the presence of NADPH and O2. This activity was significantly higher in microsomes obtained from phenobarbital (PB)-induced rats than from control microsomal preparations, whereas 3-methylcholanthrene (3-MC)-induced microsomes failed to convert l-menthol to compound II in the presence of NADPH and O2. L-Menthol elicited a type I spectrum with control (Ks = 60.6 microM) and PB-induced (Ks = 32.3 microM) microsomes whereas with 3MC-induced microsomes it produced a reverse type I spectrum (Hawthorn et al, 1988)..

One randomized 4-way crossover study was designed to determine the effect of peppermint oil and ascorbylpalmitate on cytochrome P4503A4 (CYP3A4) activity in vitro and oral bioavailability of felodipine in humans. The method was the study of the reversible mechanism-based inhibitions of nifedipine oxidation in human liver microsomes. Oral administration of 10-mg extended-release tablet of felodipine with grapefruit juice (300 mL), peppermint oil (600 mg), ascorbyl palmitate (500 mg), or water, were given to 12 healthy volunteers, and determined the pharmacokinetics of felodipine and dehydrofelodipine. The authors concluded that Peppermint oil, menthol, menthyl acetate, and ascorbyl palmitate were moderately potent reversible inhibitors of in vitro CYP3A4 activity. Nevertheless further investigation should be done (Dresser et al, 2002).

In one randomized, double blind, two way crossover study with eleven subjects, comparing the kinetics and effects of a single oral dose of Felodipine ER tablet (Plendil 10 mg), with Menthol (test) or placebo (reference), was studied the effect of menthol on the pharmacokinetics and pharmacodynamics of Felodipine in healthy subjects. The results concluded that the pharmacokinetics parameters of Felodipine and dehydrofelodipine were not markedly during the measurements (Gelal, 2002).

Excretion

The urinary metabolites result from hydroxylation at the C-7 methyl group at C-8 and C-9 of the isopropyl moiety, forming a series of mono- and dihydroxymenthols and carboxylic acids, some of which are excreted in part as glucuronic acid conjugates. Studies with tritiated I-menthol in rats indicated about equal excretion in faeces and urine. The main metabolite identified was menthol-glucuronide. Additional metabolites are mono- or di-hydroxylated menthol derivatives.

2.2.2 Assessor's overall conclusions on pharmacokinetics

The studies on the pharmacokinetics and bioavailability are few and contradictory.

In animals, peppermint is rapidly absorbed. The major biliary metabolite is menthol glucuronide, which undergoes enterohepatic circulation. After inhalation, pulmonary absorption depends on various factors and the rapid elimination indicates that there should be no accumulation during long-term application.

The urinary metabolites are excreted in part as glucuronic acid conjugates. Studies in rats indicated equal excretion in feces and urine of essential oil compounds. The main metabolite identified was menthol-glucuronide

2.3 Toxicology

2.3.1 Overview of available data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof

Toxicity

Peppermint, pulegone, menthofurane

Short-term toxicity studies demonstrated that peppermint oil (40 and 100 mg/kg b.w. day) and pulegone (80 and 160mg/kg b.w.day) induced brain lesions in rats at oral doses.

The oral LD_{50} of peppermint oil U.S.P. in fasted Wistar male rats after 24 h was found to be 4441±653 mg/kg. After 48h was 2426 mg/kg.

The interest in toxicity of pulegone, menthofuran and peppermint oil appears to have been provoked by three reports in the literature. It was reported that pulegone, when given to rats for 28 days, caused histopathological changes in the liver (vacuolisation) and the brain ("cystlike spaces") (Thorup et al. 1983a,b; Olsen and Thorup, 1984). The histopathological changes were seen in rats receiving 80 and 160 mg/kg/day of pulegone. However, all haematological and clinical chemical parameters were found to be within the normal range in all groups. There were neither obvious signs of clinical symptoms due to encephalopathy. Based on these studies the NOEL (no effect level) of pulegone was considered to be 20mg/kg bw/day. Later "confirmatory" studies by the same group, however, reported that there were no significant histopathological changes in the liver or the brain. The "cyst-like spaces" reported in the brain in the earlier studies were thus not confirmed and may have arisen from inadequate tissue fixation procedures (Molck et al. 1998). In this study the clinical biochemical examinations revealed increased plasma glucose, alkaline phosphatase and ALAT and a decreased creatinine in the dosed group. In later studies the liver toxicity of pulegone has been confirmed and a mechanism of action has been proposed based on its metabolism to menthofuran and other reactive metabolites, which are the ultimate hepatotoxins (see SCF report on Public statement on the use of HMP containing pulegone and menthofurane – EMEA/HMPC/138386/2005).

Menthone

The oral toxicity of menthone was evaluated in an animal model. The decrease in plasma creatinine and the increase in phosphatase alkaline and bilirubin were dose dependent, after levels of 0, 200, 400 and 800mg/kg b. w. /day. The NOEL for menthone in this study was lower than 200mg/kg b.w. /day (Madsen et al, 1986). A NOEL of 400 mg/kg b. w. /day was reported in a 28 day toxicity study in rats (Who, 2000).

Genotoxicity

Peppermint oil

Salmonella strains TA1537, TA98, TA1535 and TA100 at concentrations of 800, 160, 32 and 6,4 µg per plate were used to test peppermint oil. No mutagenic properties were observed[75]. Menthol and pulegone were also negative.

Peppermint oil was negative on a dose of 150µg/ml in a mouse lymphoma L5178Y TK +/- cell mutagenesis assay and, on a concentration of 155µg/ml, in an unscheduled DNA synthesis assay, on rat hepatocytes (Final report on the Safety Assessment of Mentha Piperita, 2001)

The genotoxic activity of dill, peppermint and pine essential oils were studied using chromosome aberration (CA) and sister chromatide exchange (SCE) tests in human lymphocytes in vitro and *Drosophila melanogaster* somatic mutation and recombination test (SMART) in vivo. The essential oil of *M. piperita* was shown to weakly induce SCE in a dose independent manner and to be genotoxic in the wing somatic mutation and recombination tests (SMART). Peppermint oil was the most cytotoxic and inhibited mitotic activity of human lymphocytes(Lazukta et al , 2001).

Menthone

Menthone exhibited mutagenic responses in several Salmonella tester strains, although responses were rather inconsistent in terms of concentration and requirement of S9. It was also positive in the wing somatic mutation and recombination tests (SMART) [105] and genotoxic in *D. melanogaster* ((Lazukta et al , 2001).. It was weakly positive in the host-mediated assay (mice), but not in cytogenetic or dominant lethal assays (rats). (Final report on the Safety Assessment of *Mentha piperita*, 2001)

Peppermint oil was negative in two validated tests of genotoxicity, the Ames test and the mouse lymphoma assay. Weak and inconsistent genotoxic responses in other non-validated tests are probably toxicologically inconsequential. There is more evidence for genotoxicity potential of menthol and there seems to be a discrepancy between peppermint oil and its most important constituent menthol. However, the present evidence points to a very weak or totally absent genotoxicity of peppermint oil.

Immunotoxicity

At a very high dose levels (1250mg/kg/day), peppermint did increase mortality and reduce survival time in the host resistance assay, on the rapid screening protocol, to evaluate humoral and cell-mediated immune responses (Gaworsky et al, 1994).

Phototoxicity

No effects were produced after the application of 100% peppermint oil on the back of hairless mice, irradiated with light from a fluorescent black light at an integrated UVA. The same result was obtained on a second experiment using the same protocol with two miniature swine (Final report on the Safety Assessment of *Mentha piperita*, 2001)

Teratogenicity

Menthol

No teratogenic effects were noted after oral intubations of Brazilian menthol on pregnant mice, rats, hamsters and rabbits (Food and Drug Research Labs, 1973).

Carcinogenicity

Menthol

The National Cancer Institute found no evidence of carcinogenicity after dosing Fisher 344 rats with 3750 or 7500 ppm oral dose, or $B6C3F_1$ mice with 2000 or 4000 ppm *d, l*-menthol, on a two year study, in 1979. In female mice, was noted a dose related increased deaths.

After 20 weeks of oral dosing with 1% (-) menthol, was reported a significant inhibition of induced mammary gland carcinogenesis ($p<0,001$) (Russin et al).

3 CLINICAL DATA

- For all studies cited, it should be stated by means of a detailed description which herbal substance(s)/herbal preparation(s) have been used and information should be provided for each preparation separately.

3.1 Clinical Pharmacology

3.1.1 Pharmacokinetics

3.1.1.1 Overview of available data regarding the herbal substance(s)/herbal preparation(s) including data on constituents with known therapeutic activity.

Oral administration

Peppermint oil is relatively rapidly absorbed after oral administration and eliminated mainly via the bile.

Menthol

To determine the disposition kinetics and to examine subjective and cardiovascular effects of menthol, was conducted a crossover placebo-controlled study that compared pure menthol versus placebo, along with an uncontrolled exposure to menthol in food or beverage. Twelve subjects were studied; each received a 100 mg l-menthol capsule, a placebo capsule, and 10 mg menthol in mint candy or mint tea on three different occasions. Plasma and urine levels of menthol and conjugated menthol (glucuronide), cardiovascular measurements, and subjective effects were measured at frequent intervals. Menthol was rapidly metabolized, and only menthol glucuronide could be measured in plasma or urine. The plasma half-life of menthol glucuronide averaged 56.2 minutes (95% confidence interval [CI], 51.0 to 61.5) and 42.6 minutes (95% CI, 32.5 to 52.7) in menthol capsule and mint candy/mint tea conditions, respectively ($P < .05$). The plasma area under the plasma concentration-time curve ratios for menthol capsule to mint candy/mint tea treatment averaged 9.2 (95% CI, 8.2 to 10.1) (Hadley, Gaarder, 2005).

An aqueous suspension of peppermint oil was injected along the biopsy tract in endoscopic examinations. Colonic spasm was relieved within 30 seconds in each of 20 patients using this technique (Leicester, 1982).

After administration of peppermint oil to ileostomy patients, elimination of menthol glucuronide was less than after administration to healthy subjects, indicating that part of the absorption of menthol took place in the distal small intestine.

Dermal

Using sensitive and selective gas-chromatographic methods, after skin application of camphor, menthol and methyl salicylate, the systemic absorption was examined. Concentration time profiles were erratic and variable and the half-lives relatively shorts (Martin et al, 2004).

Excretion

Pharmacokinetic studies reveal that fractionated urinary recovery of menthol is dependent on the kind of formulation used for the application of PO. Optimal pH triggered enteric coated formulations start releasing PO in the small intestine extending release over 10-12 h thus providing PO to the target organ in irritable bowel syndrome, i.e. the colon. The hypothesis is supported by anecdotal observations in patients with achlorhydria or ileostoma, respectively (Grigoleitt, 2005).

The excretion in the breast milk is undetermined.

3.1.1.2 Assessor's overall conclusions on pharmacokinetics

Peppermint oil is relatively rapidly absorbed after oral administration and eliminated mainly via the bile. Peppermint oil is highly fat soluble and rapidly absorbed at the proximal gut. However, some studies with ileostomy patients suggest that part of the absorption of menthol took place in the distal small intestine. Nevertheless pharmacokinetic studies reveal that fractionated urinary recovery of menthol is dependent on the kind of formulation used for the application of PO.

The systemic absorption after dermal application was examined and concentration time profiles were erratic and variable and the half-lives relatively shorts.

3.1.2 Pharmacodynamics

3.1.2.1 Overview of available data regarding the herbal substance(s)/herbal preparation(s) including data on constituents with known therapeutic activity.

Antispasmodic action on the smooth muscle

One study documents the relaxation of the muscles around the border from oesophagus to the stomach through peppermint oil (Anon, 1990).

One study is an investigation about peppermint oil to reduce colonic spasms during endoscopy in 20 patients. Peppermint oil is injected along the biopsy channel of the colonoscope. Colonic spasm was relieved within 30 s (Sigmund, McNally, 1969).

The principal pharmacodynamic effect of peppermint oil relevant to the gastrointestinal tract is a dose-related antispasmodic effect on the smooth musculature, as we can see on the studies presented below, due to the interference of menthol with the movement of calcium across the cell membrane. The choleretic and antifoaming effects of peppermint oil may play an additional role in medicinal use.

An aqueous suspension of peppermint oil injected along the biopsy tract in 20 patients prevented the colonic spasms that otherwise occur in endoscopic examinations (Leicester, 1982). Peppermint oil relaxes the oesophageal sphincter when administered orally (15 drops of oil suspended in 30 mL of water), eliminating the pressure differential between the stomach and oesophagus and allowing reflux to occur (Sigmund, McNally , 1969).

A randomized double-blind, double dummy, controlled trial was conducted in 100 patients to compare the antispasmodic effects of hyoscine-N-butylbromide IM, and a placebo solution administered intraluminally by the endoscope, and also the effects of a placebo solution IM with those of a peppermint oil solution administered intraluminally. The percent change in diameter of the pyloric ring before and after the administrations was defined as the the opening ratio, and the percent change in diameter between the maximally and minimally opened pyloric ring states was defined as the contraction ratio. Time until disappearance of the contraction ring(s) in the gastric antrum and side effects of the drugs were also determined. The opening ratio was significantly higher in the peppermint oil administration group than in the hyoscine-N-butylbromide injection group. The contraction ratio was lower in the peppermint group. The time required for the disappearance of the antral contraction was shorter in the peppermint oil group (97.1± 11.4) than in the hyoscine-N-butylbromide group (185.9±10.1 s; p<0,0001). No significant side effects were associated with peppermint oil, whereas such as hyoscine-N-butylbromide injection produced side effects such as dry mouth, blurred vision and urinary retention (Hiki et Al , 2003).

In nine studies, 269 healthy subjects or patients underwent exposure to peppermint oil (PO) either by topical intraluminal (stomach or colon) or oral administration by single doses or 2 weeks treatment (n = 19). Methods used to detect effects were oro-cecal transit time by hydrogen expiration, total gastrointestinal transit time by carmine red method, gastric emptying time by radiolabelled test meal or sonography, direct observation of colonic motility or indirect recording through pressure changes or relieve of colonic spasms during barium enema examination. The dose range covered in single dose studies is 0.1-0.24ml of PO/subject. With one exception, which show an unexplained potentiation of neostigmine stimulated colon activity; all other studies result in effects, indicating a substantial spasmolytic effect of PO of the smooth muscles of the gastrointestinal tract.

The effectiveness of peppermint oil added to barium sulphate suspension in relieving colonic muscle spasm during contrast barium enema examination was assessed in a double blind study with 141 patients. No residual spasm was evident in a significant proportion of patients in the treated group (60%) compared with the control group (35%). There were no adverse effects on the quality of the examination (Sparks et al, 1995).

Another comparative study, with 383 patients on DCBE (double-contrast barium contrast), with positive results from occult blood tests were performed. 4 groups, peppermint in barium, peppermint in tube, Buscopan or no treatment. In the group using peppermint oil or buscopan, the rate of patients with non-spasm examination was higher than that in no-treatment group ($p<0.0005$). Peppermint oil had the same spasmolytic effect as the systemic administration of Buscopan (n-butylscopolamine.) in the transverse and descending colon.

The pharmacodynamic study on the effect of peppermint oil (90 mg) and caraway oil on gastrointestinal motility in healthy volunteers was performed, using simultaneous determination of gastric and gall-bladder emptying and orocecal time, in comparison with placebo, cisapride and n-butylscopolamine. Peppermint oil shows a relaxing effect on the gallbladder ($P = 0.04$) and slows the small intestinal transit ($P = 0.004$) (Asao et al, 2003)

160 patients scheduled for outpatient colonoscopy were randomized in a double blind design. The objective was to determine the efficacy of peppermint oil versus placebo instillation over the ileocecal valve in the cecum, on the success rate and the duration of time required for terminal ileum intubation. The time required for TI intubation was shorter in POS group (102 seconds) than the control group (137 seconds) – $p=0,045$ (Goerg et al, 2003)**.**

3.1.2.2 Assessor's overall conclusions on pharmacodynamics

The pharmacodynamic studies demonstrated the spasmolytic effect on the smooth muscle of the intestinal tract. The different formulations may reach different target organs. An appropriate galenic formulation minimizes the adverse effects.

Peppermint oil shows activity on the relaxation of the Oddi's sphincter on the gallbladder, demonstrating some choleretic properties.

The relaxation of the oesophageal sphincter, plus the reduction in gastric and intestinal foam volume, observed in vitro, contribute to the carminative effect.

3.2 Clinical Efficacy Studies

3.2.1 Dose response studies

There are no dose-finding studies available.

The recommended dosage of 0.2 ml – 0,4ml for adults, elderly and children over 12 years (2 –3 times daily) is supported by clinical investigations as noted below, for the treatment of irritable bowel syndrome.

3.2.2 Clinical studies (case studies and clinical trials)

Oral administration

Irritable bowel syndrome

Non Controlled clinical studies

- When 50 patients suffering from irritable bowel syndrome were studied in an open multicentre trial, they received three peppermint oil capsules (of 0.2 mL) per day, each administered orally 30 minutes before a meal. Evaluation of all signs and symptoms, both pre- and post-treatment (after four weeks of treatment), confirmed a statistically significant decrease of symptoms.

Controlled clinical studies

- In two double blind, crossover studies of irritable bowel syndrome with 16 and 29 patients respectively, capsules containing peppermint oil (0.2 mL/capsule) were compared with placebo.

Patients received orally three times daily 1 or 2 capsules depending on the severity of symptoms. The overall assessment of each treatment period showed that patients felt significantly better ($p<0.01$) while taking peppermint oil capsules compared with placebo, and considered peppermint oil better than placebo in relieving abdominal symptoms ($p<0.005$).

- 34 patients with irritable bowel syndrome in whom pain was a prominent symptom were entered in a double blind clinical trial of peppermint oil (0.2 mL/capsule) versus placebo. Two capsules were taken orally three times daily. The patients' assessments at the end of two and four weeks of treatment showed no significant difference between peppermint oil and placebo in terms of overall symptoms.

- Enteric-coated capsules containing peppermint oil (0.2 mL/capsule, taken orally) were compared with placebo in a double blind, crossover trial involving 18 patients with irritable bowel syndrome. The patients received three capsules per day for 4 weeks and then changed to the alternative medication for a further 4 weeks. With peppermint oil, there was a small but statistically significant increase in frequency of defecation but no significant change in scores for global severity of symptoms or scores for the specific symptoms of pain, bloating, urgent defecation and the sensation of incomplete evacuation.

- In a double blind, crossover study, 40 irritable bowel syndrome patients were treated orally for 2 weeks with peppermint oil in enteric-coated capsules (0.2 mL/capsule), hyoscyamine (0.2 mg) or placebo. Treatment with peppermint oil tended to have a more pronounced effect on symptoms than placebo or hyoscyamine, but this was not statistically significant. (Krag,1985, Pittler, Ernst, 1998)

Reviews

On one review, 16 clinical trials in the literature search using 180-200 mg enteric-coated peppermint oil (PO) in irritable bowel syndrome (IBS) or recurrent abdominal pain in children (1 study) with 651 patients enrolled were identified. There was a prevalence of women.

Some of the studies were performed before the Rome II criteria, but according to the authors of this review, the inclusion criteria appear to be adequate. The treatment duration was from 2 to 11 weeks and in one open study was 6 months.

Nine out of 16 studies were randomized double blind cross over trials with ($n = 5$) or without ($n = 4$) run in and/or wash out periods, five had a randomized double blind parallel group design and two were open labelled studies. Placebo served in 12 and anticholinergics in three studies as comparator.

In 11 of the studies there was a daily patient rating of selected symptoms as abdominal pain, distension, flatulence, stool frequency, urgency, bloating, stool quality, frequency of attacks, severity of attacks, or the overall assessment. In two studies, the rating by patients was at intervals of two weeks. In two studies the interval was not given. In one open trial the physician rating was at the end of the week. To make this data comparable, the variable “overall success” was used (% of responders). (Grigoleit, 2005).

Study no./Ref.	Design	Study drug(s)		Treatment weeks	Patients enrolled
1 Rees (1979)	db,co,wash out period	One to two capsules t.i.d.b	Placebo 1–2 capsules t.i.d.	3/treatment	18
2 Evans et al. (1982)	db,co,randomized, wash out?	One to two capsules t.i.d.d	Placebo	2/treatment	20
3 Dew et al. (1984)	db,co,wash out period	One to two capsules t.i.d.b	Placebo 1–2 capsules t.i.d.	2/treatment	29
4 Nash et al. (1986)	db,co,no wash out, randomized	Two capsules t.i.d.a	Placebo 2 capsules t.i.d.	2/treatment	41
5 Mu¨ nst et al. (1987)	db,co,wash out, double dummy, randomized	One capsule t.i.d.a	Matching mebeverine 135 mg 1 tablet t.i.d.	3/treatment	16
6 Weiss and Ko¨ lbl (1988)	db,pg,randomized	One capsule t.i.d.a	Placebo,1 capsule t.i.d.	3	60
7 Lawson et al. (1988)	db,co,no wash out	One capsule t.i.d.b	Placebo,1 capsule t.i.d.	4	25
8 Lech et al. (1988)	db,pg,randomized	One capsule t.i.d.d	Placebo,1 capsule t.i.d.	4	47
9 Wildgrube (1988)	Matched pairs, db pg, randomized	Capsules	Matching placebo capsules	2	40
10 Carling et al. (1989)	db,3 way co, wash out	One to two capsules t.i.d.a and matching placebo	Hyoscyamine 0.2 mg and matching placebo,1–2 tablets t.i.d.	2/treatment	40
11 Schneider and Otten (1990)	db,co,wash out, randomized	One capsule t.i.d.a	Placebo 1 capsule t.i.d.	6/treatment	60
12 Fernandez (1990)	Open	One capsule t.i.d.b		4	50
13 Ambross (1990)	db,co,randomized	Not specified	Alverine citrate	11/treatment	18
14 Shaw et al. (1991)	Open, pg, randomized	One capsule t.i.d.a	Stress management program, median 6 psychotherapy sessions of each 40 min/patient	24	35
15 Liu et al. (1997)	db,pg,randomized	One capsule t.i.d. or q.i.d.a	Placebo 1 capsule t.i.d. or q.i.d.	4	110
16 Kline and Barbero (1997)	db,pg,randomized	One to two capsules t.i.d.a	Placebo 1–2 capsules t.i.d.	2	42

db ¼ double blind, co ¼ cross over, pg ¼ parallel groups. A Colpermins .
B Enteric-coated PO capsule.

C Mentacurs

D Unspecified PO formulation.

(Grigoleit, 2005).

Table 2. Summary of ‘‘overall success’’ data for peppermint (PO) oil in IBS [54-56]

Study no.	Overall success (%) peppermint oil	Comparator	Overall success comparator (%)	Comments
1	50	Placebo	13	P<0:01
2	No numerical data	Placebo	No numerical data	Overall success in favor of PO (p<0:025)
3	41	Placebo	10	P<0:001
4	39	Placebo	52	n.s.
5	No numerical data	Mebeverine	No numerical data	Except for ‘‘fullness’’ no difference between treatments
6	74	Placebo	17	P<0:001
7		Placebo		Increase in stool frequency (p<0:05),formulation problem
8	68	Placebo	26	P<0:02
9	No numerical data	Placebo	No numerical data	All symptoms improved in favour of peppermint oil (p<0:05)
10	57	Placebo Hyoscyamine	37 38	Symptom score before/after PO P<0:01; placebo and hyoscyamine p<0:05
11 12	57 93	Placebo	39	Difference n.s. p < 0:08 Open study
13	No numerical data	Alverine	No numerical data	No difference between treatments
14	18	Stress management program	72	Strongly in favour of psychotherapy after 6 months
15	79	Placebo	32	Overall success calculated from mean improvement values of symptoms, single symptoms all P<0:05
16	70	Placebo	43	Children/recurrent abdominal pain, p<0:002

Eight out of 12 placebo controlled studies show statistically significant effects in favour of PO. Average response rates in terms of "overall success" are 58% (range 39-79%) for PO and 29% (range 10-52%) for placebo. The three studies versus smooth muscle relaxants did not show differences between treatments hinting for equivalence of treatments.

A total of 71 patients dropped out. The most of them for reasons unrelated with the study. Others (n=6 worsening of symptoms, PO or placebo; n=2 nausea and vomiting by PO; n=3 perianal burning by PO; n=2 peppermint taste and pyrosis).

Adverse events reported were generally mild and transient, but very specific. PO caused the typical GI effects like heartburn and anal/perianal burning or discomfort sensations, whereas the anticholinergics caused dry mouth and blurred vision. Anticholinergics and 5HT3/4-antagonists do not offer superior improvement rates; placebo responses cover the range as in PO trials, concludes the authors[56].

The authors conclude that the clinical data in IBS reveals that peppermint oil in an enteric coated form is safe and efficacious in a sufficient number of studies, as a symptomatic remedy in a short term treatment[56].

Meta-analysis

A statistical meta-analysis of eight studies showed that the treatment of irritable bowel syndrome with peppermint oil was more effective than treatment with a placebo. It should be noted that some of the older studies had serious methodologic problems including vague inclusion criteria for patients and treatment periods that were too short. In five trials the treatment period ranged from two to four weeks and the doses were 0,2 to 0,4 ml three times daily. In three of five trials, significant benefit over placebo for global improvement were reported (p<0,001). In the descritive review, one small controlled trial suggested that stress treatment had better results than peppermint oil on a period of six months. The other two trials, placebo controlled, had or no significant improvement on pain relief or no difference from placebo. Overall, there was a significant benefit on four of the six double blind placebo controlled trials over a two to six weeks treatment period. (Pittler MH, Ernst 1998).

Dyspepsia

- A placebo controlled double-blind study has been studied in 69 woman in the treatment of abdominal distension and dyspepsia following routine gynaecological surgery, using Peppermint oil (Colpermin – Tillots Laboratories, St. Albans, Hertfordshire), in enteric coated capsules, 2 capsules, 3 times/day, during 5 days. No differences were found in abdominal distension, flatulence or abdominal pain between the two groups. Peppermint oil was not effective, but safe[61].

- In a double blind, randomized, placebo controlled, multicentre, 4-week trial, 39 patients with dyspepsia (non ulcerative), with moderate to severe pain were given a combination (Enteroplant ®) of peppermint (90mg) and caraway oil (50mg). Decrease in pain intensity was significantly greater in the treatment group (15 days-84, 2% - p=0,002; 29 days – 89, 9% - p=0,015) than in the placebo group (15 days - 50%; 29 days – 45%) (Barnick C.G., Cardozo, 1990).

Gallbladder – cholelitolytic, cholagogue, choleretic

In these clinical studies it was used a terpene preparation called Rowachol ® (Pinene 17mg, Camphene 5mg, Cineol 2mg, Menthone 6mg, Menthol 32mg, Borneol 5mg, Olive Oil 33mg – for each capsule of 100mg).

Uncontrolled study

It was given to19 of 31 patients with common bile duck stones, up to 7 capsules/day initially of Rowachol ®, and 3 capsules/day later. 8 (42%) patients had total stone disappearance in 3 to 48 months; Bile acid (chenodeoxycholic in 11, ursodeoxycholic in 4) was given also to 15, from 2 to 60 months, and within 18 months, 11 had complete stone dissolution (Somerville et al, 1985).

15 patients were treated with Rowachol ®, 3 capsules daily minimum. The treatment was effective in dissolving stones when administered in one year (Bell, Doran, 1979).

Controlled study

A human controlled study with two groups, evaluated the biliary lipid secretion and gall bladder bile composition. Rowachol ® enhanced the cholesterol solubility of gall bladder bile (*p<0,001*) and human T-tube (*p<0, 05*) bile after the ingestion of 2 capsules three times daily for 48 hours (Bell, Doran, 1979).

The Commission E monograph also describes a cholagogic action to peppermint oil.

Inhalation

Sleep/alertness action

Twenty-one healthy sleepers (11 women and 10 men) completed three consecutive laboratory sessions, to study the peppermint oil odour effect on polysomnographic sleep, alertness and mood, when presented before bedtime. Polysomnographic recordings, mood questionnaires like the Stanford Sleepiness Scale and the Profile of Mood States Questionnaire, and also Liker scales for stimulus perception, were performed. Peppermint reduced fatigue and improved mood. The subjects who rated peppermint as very intense had more total sleep than those rating it as moderately intense, showing more slow-wave sleep then in the control session. It increased NREM sleep in women, but this was not true in men, where alertness was more evident than in women. So, there are individual factors influencing the results on the physiological sleep, self-rated mood and alertness (Adam et al, 2006).

Another study examined the influence of essential oils and components (peppermint, jasmine, ylang-ylang, 1, 8-cineole and menthol) on core attention function. Six experimental groups were compared with corresponding control groups receiving water (n=20 – 4 groups; n=30 – 2 groups). The results did not reach statistical significance. The authors indicate complex correlations between subjective evaluations of substances and objective performance, concluding that the effects are mainly psychological (Ilmberger et al, 2001).

Respiratory action

According with ESCOP, inhalation of the oil for treating congestion due to common colds is believed to ease congestion, aiding respiration, by stimulating cold receptors in the respiratory tract.

A secretolytic action in the bronchi and decongestant in the nose were reported (ESCOP monographs, 2003).

Various studies did not demonstrate a change on inspiratory or expiratory nasal airway resistance, but enhances the sensation of nasal airway latency. It seems that menthol acts upon trigeminal sensory nerve endings within the nose (Eccles et al, 1988).

Postoperative Nausea

A study was performed with 18 patients in a three condition experimental design, to investigate the efficacy of peppermint oil on the relief of postoperative nausea in gynaecological surgical patients - (control group – no treatment; placebo – peppermint essence; experimental – peppermint oil), isolated from each others due to the volatile nature of the compound. The experimental group had an increased number of intra-abdominal procedures, received more opioid analgesia postoperatively and required less traditional antiemetics (Tate S, 1997)

External application

Tension headache

There is not clear clinical and pharmacological data to support this indication, but there are some studies, which enable an assessment of Peppermint oil for external use in tension headache, as follows.

Controlled studies

The analgesic effect of peppermint oil (10% in ethanol) was investigated in 32 healthy subjects in a double blind placebo-controlled, randomized, four-fold crossover study. Neurophysiological, psychological and experimental algesimetric parameters were investigated. Four different test preparations were applied to large areas of the forehead and temples using a small sponge. Preparations containing peppermint with or without *Eucalyptus* were superior in pain reduction and had a muscle relaxing and mentally relaxing effect.

Compared to the application of placebo, 10% peppermint oil in ethanol solution significantly reduced the clinical headache intensity already after 15 minutes ($p < 0.01$). This significant clinical reduction of the pain intensity continued over the one-hour observation period (Dresser, 2002).

The effect of a locally applied peppermint oil preparation on tension-type headache was examined in the design of a randomized, placebo-controlled double-blind crossover study. The preparation was tested against both the reference substance acetaminophen and to the corresponding placebo. The liquid test preparation contained 10 g of peppermint oil and ethanol (90%) ad 100 (test preparation LI 170, Lichtwer Pharma, Berlin); the placebo was a 90% ethanol solution to which traces of peppermint oil were added for blinding purposes. The reference preparation contained 500 mg acetaminophen; the placebo tablet was identical to the verum in size and appearance. The study included the analysis of 164 headache attacks of 41 patients of both sexes ranging between 18 and 65 years of age, suffering from tension-type headache in accordance with the IHS classification. Compared to the application of placebo, 10% peppermint oil in ethanol solution significantly reduced the clinical headache intensity already after 15 minutes ($p < 0, 01$). This significant clinical reduction of the pain intensity continued over the one hour observation period. Acetaminophen, too, proved to be efficient compared to placebo ($p < 0, 01$). There was no significant difference between the efficacy of 1,000 mg of acetaminophen and 10% peppermint oil in ethanol solution.

The topical application of peppermint oil produces a prolonged cold sensation at the local of application, by the stimulation of the cold-sensitive receptors, giving an analgesic effect.

The application to the forehead showed on the EMG activity, a significant reduction of the M temporalis wave, as a pronounced increase in blood flow through the capillaries of the skin (Fachinfo Euminz, 1997)

Safety data were available for 150 Patients without AE´s.

Analgesic effect

Case study

Report of a post herpetic neuralgia on a 76 years woman, with relief of the pain during 4-6 hours after the local application of peppermint oil (containing 10% menthol). During two months of treatment she continued to feel the same effect (Davies et al, 2002).

3.2.3 Clinical studies in special populations (such as elderly and children)

Clinical studies in children

In a randomized, double-blind controlled trial of two weeks, 42 children (8 to 17 years old) with irritable bowel sd were given ph dependent enteric coated peppermint oil capsules, versus placebo. The patients weighing more than 45kg received 2 capsules, 3 times a day. The smaller children, who weighed 30Kg to 45 kg, received 1 capsule 3 times a day. After two weeks, 75% of those receiving peppermint oil, reduced severity of pain associated with the IBS, but not the other symptoms, like heartburn, gas, urgency of stools, belching, stool pattern or stool consistency. No adverse events were reported (Kline Robert et al, 2001).

3.2.4 Assessor's overall conclusions on clinical efficacy

Oral administration

IBS

The Rome II diagnostic criteria of Irritable Bowel Syndrome always presumes the absence of a structural or biochemical explanation for the symptoms and is made only by a physician.

Irritable Bowel Syndrome can be diagnosed based on at least 12 weeks (which need not be consecutive) in the preceding 12 months, of abdominal discomfort or pain that has two out of three of these features*:*

1. Relieved with defecation; and/or
2. Onset associated with a change in frequency of stool; and/or
3. Onset associated with a change in form (appearance) of stool.

Other symptoms that are not essential but support the diagnosis of IBS:

- Abnormal stool frequency (greater than 3 bowel movements/day or less than 3 bowel movements/week);
- Abnormal stool form (lumpy/hard or loose/watery stool);
- Abnormal stool passage (straining, urgency, or feeling of incomplete evacuation);
- Passage of mucus;
- Bloating or feeling of abdominal distension

It affects more women than men and is more common in patients 30 to 50 years of age (Hadley et al, 2005)

According to the document "Points to Consider on the Evaluation of Medicinal Products for the Treatment of Irritable Bowel Syndrome" (CPMP/2003), considering the chronic character of this disease, it may be acceptable to conduct a number of studies with different designs to provide all the required efficacy data (dose response studies, efficacy with first use – 4 weeks duration, withdrawal/rebound effect, efficacy with repeated use). The trials must be long, considered as necessary 6 months of active treatment. Other studies should be justified. On short term studies of 4 weeks, would be required a 50% of the time on the response on the specified improvement in symptoms.

IBS is a complex disorder that affects many patients. Its treatment is also complex, because a variety of processes appear to be involved. So, it is difficult to find treatment suitable for all sort of IBS patients, but effective towards specific aspects. IBS is a chronic condition, with unpredictable periods of exacerbation and remission. Thus, clinical trials of only few weeks are of limited relevance to conclude about the effectiveness of the treatment.

Some studies in the literature show methodological problems, as use of no validated scales, the randomization procedure is not clear, there is lack of adequate washout period, limited treatment period (2-4 weeks), have small sample sizes and unclear diagnostic criteria. Despite this, some interventions with peppermint oil have been shown to be clinically effective in the treatment of symptoms of IBS, in several randomized well designed controlled trials. A variety of outcome measures have been used, making it difficult to compare the results of the trials.

The meta-analysis by Pittler and Ernst reported that the role of peppermint oil in the symptomatic treatment has not been established and more studies are needed to clarify the issue.

Nevertheless, the clinical studies demonstrated a reduction in spasms during barium enemas and endoscopies, as smooth muscle relaxing properties, pointing peppermint oil as an antispasmodic agent on the GI tract, reducing abdominal pain. The enteric-coated capsules are generally recommended to reach the target organ and avoid undesirable effects like heartburn and oesophageal reflux.

The carminative properties attributed to peppermint were documented by the literature, helping to relief the flatulence.

Dyspepsia (non-ulcer)

Small number of controlled trials with a combination of peppermint and caraway oil shows some benefits on dyspepsia symptoms. It is not clear what constituent is the most effective.

Antispasmodic

During endoscopy and colonoscopy, the topical intraluminal administration of peppermint oil, was used as antispasmodic agent in several studies, with superior efficacy than placebo and also than hyoscine-N-butylbromide, with less adverse reactions.

Cholagogic, cholelitolytic, and choleretic

Some studies appointed cholagogic, cholelitolytic, and choleretic properties, but some more trials are necessary, with a better design.

Tension headache

According to the IHS classification (ICHD-II) - International Headache Society 2003, tension type headache is classified as:

2. Tension-type headache (TTH)

2.1 Infrequent episodic tension-type headache

2.1.1 Infrequent episodic tension-type headache associated with pericranial tenderness

2.1.2 Infrequent episodic tension-type headache not associated with pericranial tenderness

2.2 Frequent episodic tension-type headache

2.2.1 Frequent episodic tension-type headache associated with pericranial tenderness

2.2.2 Frequent episodic tension-type headache not associated with pericranial tenderness

2.3 Chronic tension-type headache

2.3.1 Chronic tension-type headache associated with pericranial tenderness

2.3.2 Chronic tension-type headache not associated with pericranial tenderness

2.4 Probable tension-type headache

2.4.1 Probable infrequent episodic tension-type headache

2.4.2 Probable frequent episodic tension-type headache

2.4.3 Probable chronic tension-type headache

This kind of primary headache is very common, ranging from 30 to 78% in several studies. It was first considered as psychogenic, but recent studies suggest a neurobiological basis, especially for the more severed cases. The last edition of *The International Classification of Headache Disorders* subdivided episodic tension-type headache further, into an *infrequent* subtype with headache episodes less than once per month and a *frequent* subtype. Another difference in this edition is related with the disorder of the precranial muscles, using now for the subdivision the tenderness on manual palpation and not the surface EMG or pressure algometry (The International Classification of Headache Disorders, 2004).

This indication is mentioned in the ESCOP monograph. The Commission E monograph only includes the indication, muscle and neuralgic complaints“

The peppermint oil, by laboratory tests, seems to exert some actions on mechanisms associated with the pathophysiology of tension headache, producing an analgesic affect, after administering a 10% solution on the forehead and the temples of the patients.

The comparative clinical study with 1,000 mg acetaminophen, demonstrated no significant difference between both products on the relief of the pain. The numbers of patients in the studies were small; the inclusion criteria are not well defined with a large range of ages. The characteristics of the pain described – 4,99 days per month for 14,12 years – fulfil the point A of the diagnostic criteria of the *Frequent episodic tension-type headache* (ICHD-II) and Episodic tension-type headache (IHS code).

More research is needed to conclude about the effectivity on this indication. In Finland the indication "herbal medicinal product for temporary headache" is authorized since 2003.

Also for the relief of headache, for adults and children over 6 years, a local application (100% oil) of the forehead with the aid of an applicator several times at intervals of 15 minutes, is proposed (Germany – MA, 1978)

3.3 Clinical Safety / Pharmacovigilance

3.3.1 Patient exposure

Peppermint essential oil widely used in flavouring, cosmetic formulations and skin-conditioning agent. In general is considered as safe ingredient for use in dietary supplements and common as a folk medicine.

The FDA calculated the estimated human exposure from cosmetic use, based on the concentration of use information supplied by industry. Using a body splash product containing 0.2% Peppermint Oil and assuming 100% absorption over a body surface of 17,000 cm2 and a daily application of 1 mg/cm2 (»17 ml of the product), the FDA estimated an exposure of 34 mg/day. For a 60-kg person, this amounted to an estimated daily dose of 0.6 mg/kg/day (FDA 1997) (Final report on the Safety Assessment of *Mentha Piperita*, 2001).

The highest recommended daily dose in EU is 1,2 ml peppermint oil i.e. 1080 mg peppermint oil, which contains maximum 140mg pulegone +menthofurane (Ph Eur). For a 60Kg person this would correspond to a daily intake of 2.3 mg/kg

Menthol

In 1976, FAO/WHO Joint Expert Committee on Foods Additives established an ADI of 0, 2 mg/kg body weight/day for menthol. On 2000, an ADI of 0-4mg/kg of body weight/day was allocated (WHO 2000.

Pulegone and menthofurane

Maximum levels for pulegone in foodstuff and beverages to which flavourings or other food ingredients with flavouring properties have been added: 25 mg/kg in foodstuff, 100 mg/kg in beverages, with the exception of 250 mg/kg in peppermint or mint flavoured beverages and 350 mg/kg in mint confectionery (Annex II of Directive 88/388/EEC). Pulegone may not be added as such to foodstuff. Committee of Experts on Flavouring Substances (CEFS) of the Council of Europe (1997): Menthofurane is the proximate hepatotoxin of pulegone. Tolerated daily intake (TDI) of menthofurane and pulegone was set to 0.1 mg/kg bw, based on a no effect level (NOEL) of 20 mg/kg bw/d in the 28 days oral toxicity study in rats (Thorup et al. 1983 a,b) with a safety factor of 200. Menthofurane is listed in the register of chemically defined flavouring substances laid down in Commission Decision (1999/217/EC, 2002/113/EC).

USA: Pulegone and menthofurane have FEMA GRAS status and are listed among the authorized synthetic flavouring substances. JECFA (Joint FAO/WHO Expert Committee on Food Additives, 2000): "No safety concern" was applied to (R)-(+)-pulegone and structurally related flavouring agents including (R)-(+)-menthofurane.

3.3.2 Adverse events

A total of 213 patients and healthy volunteers have been included in 8 studies where efficacy and safety in the use of peppermint oil were investigated. Oral administration in capsules or direct injection into the colon varied from a single dose to two and four weeks of treatment at daily doses of 3-6 x 0.2 mL of the oil. Peppermint oil, at concentrations of 20-50 μg/ml, evoked ion permeability of heart cell membranes.

PO caused the typical GI effects like heartburn and anal / perianal burning or discomfort sensations in a literature search; 16 clinical trials investigating 180–200 mg enteric-coated peppermint oil (PO) in irritable bowel syndrome (IBS) or recurrent abdominal pain in children (1 study) with 651 patients enrolled were identified (Grigoleit , 2005).

Adverse effects were reported in six trials, in the vero treatment, like heartburn, perianal burning blurred vision, nausea and vomiting. The frequency ranged from 11% to 36% (**Pittler, Ernst, 1998).**

Menthol

A case of asthma due to menthol is reported in a 40-year-old woman with no history of asthma or any other allergy. The aetiology was suggested by the history of exposure. The diagnosis was confirmed by the clinic history as by skin tests (Santos, 2001)

A form of stomatites and glossites with extremely prominent circumvallated papillae in patients who consumed excessive amounts of mint-flavoured sweets was described (Santos, 2001)

Pulegone

A literature review of cases of human intoxication with pennyroyal oil (pulegone content 62-97%) indicate that ingestion of 10 ml (corresponding to ca 5.4-9 g pulegone, ca 90-150 mg/kg bw for a 60 kg person; calculated with a relative density of 0.9 as for peppermint oil) resulted in moderate to severe toxicity and ingestion of greater than 15 ml (corresponding to ca 8-13 g pulegone, ca 130-215 mg/kg bw for a 60 kg person) resulted in death. The clinical pathology was characterised by massive centrilobular necrosis of the liver, pulmonary edema and internal haemorrhage (SCF, 2002). A non-urgent information request was sent out to the member states concerning use and association of licensed herbal medicinal products containing pennyroyal oil, peppermint oil and mint oil with reports of liver damage.

The highest recommended daily dose in EU is 1.2 ml peppermint oil i.e. 1080 mg peppermint oil, which contains maximum 140 mg pulegone + menthofurane (Ph Eur). For a 60 kg person this would correspond to a daily intake of 2.3 mg/kg bw. Clearly, this recommended daily dose of peppermint oil in herbal medicinal products results in an intake of pulegone/menthofurane that exceeds the TDI (0.1 mg/kg) set for food by CEFS.

No certain cases of liver damage caused by peppermint oil or mint oil were reported (EMEA/HMPC/138386/2005).

Inhalation

Some reports about auricular fibrillation after the inhalation and ingestion of excessive amounts of mentholated products were published in medical journals (The Lancet, 1962)

Inhalation of large doses of menthol was reported to cause dizziness, confusion, muscle weakness, nausea or double vision (Natural Standard Research Collaboration, 2005).

3.3.3 Serious adverse events and deaths

Anaphylactic shock is reported (Germany).

3.3.4 Laboratory findings

Not relevant

3.3.5 Safety in special populations and situations

3.3.5.1 Intrinsic (including elderly and children) /extrinsic factors

Contact sensitivity

Report of 12 cases of contact sensitivity to the flavouring agents, menthol and peppermint oil, in patients presenting with intra-oral symptoms in association with burning mouth syndrome, recurrent oral ulceration or a lichenoid reaction. The patients were referred from the Glasgow Dental Hospital over a 4-year period for assessment of the possible contribution of contact sensitivity to their complaints. 5 patients with burning mouth syndrome demonstrated contact sensitivity to menthol

and/or peppermint, with 1 patient sensitive to both agents, 3 positive to menthol only and 1 to peppermint only. 4 cases with recurrent intra-oral ulceration were sensitive to both menthol and peppermint. 3 patients with an oral lichenoid reaction were positive to menthol on patch testing, with 2 also sensitive to peppermint. 9 of the 12 cases demonstrated additional positive patch test results. After a mean follow-up of 32.7 months (range 9-48 months), of the 9 patients that could be contacted, 6 patients described clearance or improvement of their symptoms as a consequence of avoidance of menthol/peppermint (Goel, Lao, 2005).

Positive reactions were observed in 7 of 450 dermatitic patients tested with a patch of 2% Peppermint oil in yellow soft paraffin. Other study revealed reaction on in 6 of 86 dermatitic patients (Ernst, 2000).

Clinical dermal testing demonstrated that 8% Peppermint oil was not a sensitizer and 2% gave a small number of positive reactions in dermatitic patients (Final report on the Safety Assessment of *Mentha piperita*, 2001).

There are some reports referring allergic contact dermatitis after topical application on the skin of peppermint oil. These reactions are the most of the time transient and of mild to moderate sensivity (Ernst, 2000).

Use in children

The nasal mucosa is an autonomic reflexogen organ, which has a distance action to the heart, lungs and circulation and may lead to sudden apnoea and glottal constriction. The children less than 2 years old present particularly this reflex, so all the substances with a strong odour must be avoided (Dost., Leiber, 1966).

The occurrence of jaundice in babies exposed to menthol is mentioned in one report at the Medline, advising patients with G6PD deficiency to use menthol cautiously (Natural Standard Research Collaboration, 2005).

According to the proposal of SPC for herbal medicinal products containing the essential oils Eucalyptus oil, Peppermint oil, Mint oil and Camphor, Cineol, Menthol, the product should not be used in children under the age of 2 years and in children with a history of seizures (febrile or not).

3.3.5.2 Drug interactions

Peppermint oil used on the skin with 5-fluouracil may increase the absorption rate of 5-fluouracil.

Use of food or antacids administered at the same time could cause early release of capsule content, if this is the pharmaceutical form used. Other medicinal products used for the normalization of the digestive function, should be avoided.

3.3.5.3 Use in pregnancy and lactation

Safety during pregnancy and lactation has not been established. As a precautionary measure, because of lack of data, use during pregnancy and lactation is not recommended.

3.3.5.4 Overdose

In animal studies, at 40 and 100 mg/kg body weight/day dose levels, histopathological changes in the cerebellum white matter were seen.

Overdose may cause severe gastro-intestinal symptoms, diarrhoea, epileptic convulsions, loss of consciousness, apnoea, nausea and disturbances in cardiac rhythms, ataxia and other CNS problems, probably due to the presence of menthol.

In the event of over usage, the stomach should be emptied by gastric lavage. Observation should be carried out with symptomatic treatment if necessary.

Inhalation of larges doses of menthol may lead to dizziness, confusion, muscle weakness, nausea and double vision (Natural Standard Research Collaboration, 2005).

3.3.5.5 Drug abuse

One case of fulminant pulmonary oedema following IV injection of 5 ml of peppermint oil was described, on a patient with history of drug abuse (Matthias B. et al. 2005)

3.3.5.6 Withdrawal and rebound

Not relevant

3.3.5.7 Effects on ability to drive or operate machinery or impairment of mental ability

Not relevant

3.3.5.8 Contraindications

Hypersensitivity to peppermint and menthol.

Oral application

People with chronic heartburn, severe liver damage, and inflammation of the gallbladder, obstruction of bile ducts and other occlusive disorders of the GI tract should avoid it (Matthias B. et al. 2005)

People with gallstones should consult a physician before using peppermint oil (Sigmund DJ, McNally, 1969)

External application

Open skin areas of small children, especially on the nose, face and chest, are not recommended. Children under 2 years of age.

3.3.6 Assessor's overall conclusions on clinical safety

The adverse events reported were generally mild and transient, in the doses recommended for the therapeutic indications, in non-allergic adults.

When used orally, it may cause heartburn, perianal burning, blurred vision, nausea and vomiting. Heartburn is related with the release of the oil in the upper GI tract, which relaxes the lower oesophageal sphincter, facilitating the reflux. The same occurs in the cases of hiatal hernia. This particular undesirable effect is minimized by an appropriate pharmaceutical formulation.

People with gallbladder disease, severe liver damage, gallstones and chronic heartburn should avoid the intake of peppermint oil.

Menthol and peppermint oil caused burning mouth syndrome, recurrent oral ulceration or a lichenoid reaction, by contact sensitivity in the intra-oral mucosa, in sensitive patients.

When applied on the skin, it may cause allergic reactions, as skin rashes, contact dermatitis and eye irritation.

Use in infants or children is not recommended, when inhaled, taken by mouth or if applied on open skin areas, on the face or chest, due to the potential toxicity of the product.

Because there is a lack of information about the safety during pregnancy and breastfeeding, the use is not recommended**.**

. In animals (rats), peppermint oil increases levels of cyclosporine in the blood, but this is not clear in humans.

On laboratory studies, peppermint oil is a moderately potent reversible inhibitor of in vitro CYP3A4 activity. The levels of drugs and supplements, which are processed by this enzyme, may be increased.

4. ASSESSOR'S OVERALL CONCLUSIONS

Peppermint oil has been use for generations as a digestive and carminative. More recently, as an authorized medicinal product for oral use, has been prescribed under the approved indication for the symptomatic relief of the irritable bowel syndrome. It has been also used topically, as a medicinal product, for the symptomatic treatment of neuralgic pain, in mild to moderate tension headache and for the relief of symptoms in cough and colds.

There are a lack of clinical studies to conclude about the efficacy of peppermint oil on the treatment of dyspepsia and on the treatment of cough and colds.

According to the preclinical and clinical data assessed and presented on this report, peppermint oil demonstrated an antispasmodic action of the smooth muscle of the GI tract, relieving minor spasms, flatulence and abdominal pain.

In general, the safety clinical studies showed transient and mild adverse effects. To minimise the adverse effects, like heartburn, the enteric-coated tablets are recommended. Some interactions were reported in vitro and in vivo studies, but more research should be done.

The peppermint oil, by laboratory tests, seems to exert some actions on mechanisms associated with the pathophysiology of tension headache, producing an analgesic affect, after administering a 10% solution on the forehead and the temples of the patients. The clinical studies are small but the results demonstrated the efficacy of peppermint oil on the episodic tension-type headache, according to the IHS classification. More research is needed to confirm these studies.

Nevertheless, this kind of indication needs the diagnosis of a medical doctor and must not be considered as a traditional medicinal product.

The indications proposed considered as proven, for well-established use are:

- Oral use

1. Herbal medicinal product for the symptomatic relief of minor spasms of the gastrointestinal tract, flatulence and abdominal pain, especially in patients with irritable bowel syndrome.

- Cutaneous use

2. Herbal medicinal product for the symptomatic relief of mild tension type headache.

ANNEXES

PROPOSED COMMUNITY HERBAL MONOGRAPHS FOR MENTHA X PIPERITA L., AETHEROLEUM

III. ASSESSMENT REPORT FOR HERBAL SUBSTANCE(S), HERBAL PREPARATION(S) OR COMBINATIONS THEREOF WITH TRADITIONAL USE

***Mentha x piperita* L., aetheroleum**

BASED ON ARTICLE 16D(1) AND ARTICLE 16F AND 16H OF DIRECTIVE 2001/83/EC AS AMENDED

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Mentha x piperitae* L., aetheroleum
Herbal preparation(s)	Menthae piperita aetheroleum
Pharmaceutical forms	Liquid dosage forms
Rapporteur	Dr Helena Pinto Ferreira Dr Ana Paula Martins

1 INTRODUCTION

Peppermint is an herbaceous plant highly aromatic, yielding a valuable essential oil widely used in flavouring, medicine and toiletries. Native to Europe, peppermint was much used to ancient times, having a long history of medicinal use, dating to ancient Egypt, Greece and Rome. Peppermint oil has been used historically for several health conditions, such as common cold conditions, cramps, headache, indigestion, joint pain and nausea, given orally or topically.

1.1 Description of the traditional herbal substance(s), herbal preparation(s) or combinations thereof

- Herbal substance(s)[4] [5]:

Mentha x piperitae L., aetheroleum

- Herbal preparation(s)[1] [2]:

Menthae piperita aetheroleum

2 TRADITIONAL MEDICINAL USE

- It should be stated by means of a detailed description which herbal substance(s)/herbal preparation(s) have been used and information should be provided for each preparation separately.

2.1 Information on period of medicinal use in the Community regarding the specified indication

See point 2.3.1

2.2 Type of tradition, where relevant

European phytotherapy.

Ayurvedic medicine.

2.3 Bibliographic/expert evidence on the medicinal use

It is not certain if peppermint oil was produced in the Middle Ages (Gildemeister and Hoffman, 1900). One of the oldest specimens of peppermint is included in the herbarium of the English botanist John Ray (1628-1705).

There are reports of pharmacological and clinical studies published in medical, pharmacological and toxicological Journals since 1941. Oswald, N.C., in the British Medical Journal, concludes that the most desirable property of menthol is their pleasant smell because the main virtue of steam inhalation "is the expectorant effect of hot, moist hair".

According to Commission E, the uses proposed are:
Internal: Spastic discomfort of the upper gastrointestinal tract and bile ducts, irritable colon, catarrhs of the respiratory tract, inflammation of the oral mucosa.
External: Myalgia and neuralgia.

[4] According to "Note for guidance on Quality of herbal medicinal products" (CPMP/QWP/2819/00…)
[5] According to "Note for guidance on Specifications: Test procedures and acceptance criteria for herbal drugs, herbal preparations and herbal medicinal products" (CHMP/QWP/2820/00)

From ESCOP:
Internal use: Symptomatic treatment of digestive disorders such as flatulence; irritable bowel syndrome; symptomatic treatment of coughs and colds.
External use: Relief of coughs and colds; symptomatic relief of rheumatic complaints; tension-type headache; pruritus, urticaria and pain in irritable skin conditions.

There is a reference on Martindale The Extra Pharmacopoeia, 27th Edition, June 1977, that refers peppermint oil as "an aromatic carminative, relieving gastric and intestinal flatulence and colic and is employed with purgatives to prevent griping".

On the Indian Materia Medica by Dr K. M. Nadkarni's (3rd revised edition – 1976, reprinted 1999), peppermint is referred as a powerful anodyne, anaesthetic, antiseptic and germicide used in herpes zoster, pruritus; for congestive headaches, rheumatism and neuralgia; indicated also for toothache caused by caries, and as an antiseptic for inhalation.

2.3.1 Evidence regarding the indication/traditional use

External use

1. For the relief of coughs and colds – WHO monographs; Germany 1978 (marketing authorization)
2. For symptomatic relief of muscle pain and of neuralgic pain, for example in mild to moderate tension headache – Germany – 1978, 1983 (marketing authorization)
3. Pruritus, urticaria and pain in irritable skin conditions – ESCOP monograph 2nd edition
4. Myalgia and neuralgia - Commission E Monographs

For inhalation

5. For the relief of symptoms in coughs and colds – Germany – 1978, 1983 (marketing authorization)

Traditionally used in cases of nasal congestion and common cold – France, Traditional Use 2005

6. Herbal medicinal products to treat symptoms of cold - Finland (marketing authorization, March 2003))

Oral use

7. Symptomatic treatment of coughs and colds – ESCOP monograph 2nd edition

   Treat symptoms of cold – Finland (marketing authorization, March 2003)

   Catarrhs of the respiratory tract, inflammation of the oral mucosa – Commission E Monographs

   Traditionally used locally (oromucosal spray solutions, lozenges) as an analgesic in conditions of the oral cavity and/or pharynx. - France, Traditional Use, 2005

8. Herbal medicinal product to balance mild, temporary and functional disorders in digestive tract - Finland (marketing authorization, March 2003)

On Ayurvedic medicine (Pudine, paparaminta):

External use: For muscle and joint stiffness

For cold, flu – *kapha*

2.3.2 Evidence regarding the specified strength

Peppermint oil should be used with caution. Doses of menthol over 1 g/Kg b.w. may be deadly.

The data from **Germany**, on the authorized products is mentioned:

Indication 2, 5% - 100% essential oil - cutaneous liquid

From **Martindale The Extra Pharmacopoeia**, 27th Edition, June 1977:

Peppermint water (U.S.N.F) – a saturated solution of peppermint oil in water.

Peppermint spirit (B.P.C.) – Spiritus Menthae Piperitae; Peppermint oil 10 ml, alcohol (90%) to 100 ml. Dose: 0, 3 to 2 ml.

From **Commission E Monographs**:

External use: Semi-solid and oily preparations 5-20%

In aqueous-ethanol preparations 5-10%

In nasal ointments 1-5% essential oil.

From **ESCOP**

External use:

Indication 3 – In dilute liquid or semisolid preparations equivalent to 1,1 – 1,0% m/m menthol or 1.25 – 16% m/m menthol.

- Children 4-10 years

Semi-solid preparations 2 -10% ; hydroethanolic preparations 2-4%

- Children 10-16 years

Semi-solid preparations 5 - 15% ; hydroethanolic preparations 3 – 6%

2.3.3 Evidence regarding the specified posology

For inhalation:

3 or 4 drops of the oil added to hot water, up to three times daily (Germany - authorized medicinal products, ESCOP, Commission E monographs)

2-3 drops spread on a stick and inhale – not more than three times/da**y-** Finland (marketing authorization, March 2003) - not recommended for children under 12 years old.

4 x daily 4 spray nasal (2 in each nostril) or 4 buccal spray for adults and children over 6 years – France (TMP)

3-4 drops in hot water - Commission E Monographs

For oral use:

6 – 12 drops daily, that means: 2 – 3 times daily 3-4 drops - Germany (authorized medicinal products):

2-3 drops (0,08-0,12 ml) 3-4 times per day (0,2 – 0-5 ml) – Finland (marketing authorization, March 2003) - not recommended for children under 12 years old.

External use:

100% peppermint oil – some drops locally applied with the aid of an applicator several times at intervals of 15 minutes - Germany (authorized medicinal products)

Peppermint oil in ethanol solution in an applicator - Germany (authorized medicinal products)

2.3.4 Evidence regarding the route of administration

See point 2.3

2.3.5 Evidence regarding the duration of use

Finland – not recommended to use this product continuously over three months time.

Because of safety concerns the duration must be limited. If the symptoms persist during the treatment a medical doctor must be consulted.

2.4 Assessor's overall conclusion on the traditional medicinal use

Peppermint oil had been used for a long time as a medicine, orally, topically and for inhalation. There are sufficient data to demonstrate its traditional use for several indications, with more than 15 years in the EU countries, as more than 30 years on others.

2.5 Bibliographic review of safety data of the traditional herbal medicinal substances

2.5.1 Patient exposure

2.5.2 Adverse events

See point 3.3.2

2.5.3 Serious events and deaths

See 3.3.3

2.5.4 Intrinsic (including elderly and children)/extrinsic factors

See point 3.3.5.1

2.5.5 Drug-drug interactions and other interactions

Peppermint oil used on the skin with 5-fluouracil may increase the absorption rate of 5-fluouracil.

2.5.6 Use in pregnancy and lactation

2.5.7 Overdose

Inhalation of larges doses of menthol may lead to dizziness, confusion, muscle weakness, nausea and double vision [107].

For oral mucosal use see point 3.3.5.4.

2.5.8 Drug abuse

2.5.9 Withdrawal and rebound

Not relevant

2.5.10 Effects on ability to drive or operate machinery

Not relevant

2.5.11 Contra indications (hypersensitivity and allergic potential to be both covered)

It is contraindicated in cases of hypersensitivity to peppermint oil.

Use in children under 2 years old, because menthol can induce reflex apnoea and laryngospasm.

In children with history of seizures (febrile or not).

2.6 Non-clinical safety data

2.6.1 Overview of available data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

(e.g. single/repeat dose toxicity, genotoxicity, carcinogenicity and reproductive and developmental toxicity, local tolerance, other special studies)

Assessor's overall conclusions on safe use

It is contraindicated in cases of hypersensitivity to peppermint oil and in children under the age of two years old because menthol can induce reflex apnoea and laryngospasm.

In children with history of seizures (febrile or not).

3 PHARMACOLOGICAL PROPERTIES[6]

3.1 Overview of pharmacological effects of herbal substance(s), herbal preparation(s) and relevant constituents thereof on the basis of long-standing use and experience

4 LITERATURE REFERENCES

5 ASSESSOR'S OVERALL CONCLUSIONS

Peppermint oil has been used historically for several health conditions, orally, topically and for inhalation, existing in countries of the EU as medicinal products with marketing authorization. The oral use for digestive complaints was subject to several pharmacological and clinical studies, giving sufficient data to be considered with a well-established use for the indication "Symptomatic relief of minor spasm of the gastrointestinal tract, flatulence and abdominal pain, experienced by patients with irritable bowel syndrome".

The indications proposed, which demonstrated traditional use and plausibility, according to the pharmacological properties, are the following:

External use:

I) For the relief of symptoms in coughs and colds;
II) For symptomatic relief of localized muscle pain,
III) For the symptomatic relief of localised pruritic conditions in intact skin.
Inhalation:
IV) For the relief of symptoms in coughs and colds.
Oramucosal use
V) For the relief of symptoms in coughs and colds

ANNEXES

PROPOSED COMMUNITY HERBAL MONOGRAPHS ON MENTHA X PIPERITA L., AETHEROLEUM

LITERATURE REFERENCES

[6] Not required as per Article 16c(1)(a)(ii) of Directive 2001/83/EC as amended

STATEMENT

ADOPTED: 21 June 2017

doi: 10.2903/j.efsa.2017.4908

Risks for human health related to the presence of pyrrolizidine alkaloids in honey, tea, herbal infusions and food supplements

EFSA Panel on Contaminants in the Food Chain (CONTAM),
Helle Katrine Knutsen, Jan Alexander, Lars Barregård, Margherita Bignami, Beat Brüschweiler, Sandra Ceccatelli, Bruce Cottrill, Michael Dinovi, Lutz Edler, Bettina Grasl-Kraupp, Christer Hogstrand, Laurentius (Ron) Hoogenboom, Carlo Stefano Nebbia, Isabelle P. Oswald, Annette Petersen, Martin Rose, Alain-Claude Roudot, Tanja Schwerdtle, Christiane Vleminckx, Günter Vollmer, Heather Wallace, José Angel Ruiz Gomes and Marco Binaglia

Abstract

EFSA was asked by the European Commission to deliver a scientific opinion on the risks for human health related to the presence of pyrrolizidine alkaloids (PAs) in honey, tea, herbal infusions and food supplements and to identify the PAs of relevance in the aforementioned food commodities and in other feed and food. PAs are a large group of toxins produced by different plant species. In 2011, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) assessed the risks related to the presence of PAs in food and feed. Based on occurrence data limited to honey, the CONTAM Panel concluded that there was a possible health concern for those toddlers and children who are high consumers of honey. A new exposure assessment including new occurrence data was published by EFSA in 2016 and was used to update the risk characterisation. The CONTAM Panel established a new Reference Point of 237 μg/kg body weight per day to assess the carcinogenic risks of PAs, and concluded that there is a possible concern for human health related to the exposure to PAs, in particular for frequent and high consumers of tea and herbal infusions. The Panel noted that consumption of food supplements based on PA-producing plants could result in exposure levels causing acute/short-term toxicity. From the analysis of the available occurrence data, the CONTAM Panel identified a list of 17 PAs of relevance for monitoring in food and feed. The Panel recommended continuing the efforts to monitor the presence of PAs in food and feed, including the development of more sensitive and specific analytical methods. A recommendation was also issued on the generation of data to identify the toxic and carcinogenic potency of the PAs commonly found in food.

Keywords: pyrrolizidine alkaloids (PA), origin, chemistry, analysis, exposure, risk assessment, margin of exposure

Requestor: European Commission

Question number: EFSA-Q-2016-00800

Correspondence: contam@efsa.europa.eu

Panel members: Jan Alexander, Lars Barregård, Margherita Bignami, Beat Brüschweiler, Sandra Ceccatelli, Bruce Cottrill, Michael Dinovi, Lutz Edler, Bettina Grasl-Kraupp, Christer Hogstrand, Laurentius (Ron) Hoogenboom, Helle Katrine Knutsen, Carlo Stefano Nebbia, Isabelle P. Oswald, Annette Petersen, Martin Rose, Alain-Claude Roudot, Tanja Schwerdtle, Christiane Vleminckx, Günter Vollmer and Heather Wallace.

Acknowledgements: The Panel wishes to acknowledge all European competent institutions, Member State bodies and other organisations that provided data for this scientific output.

Suggested citation: EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Knutsen HK, Alexander J, Barregård L, Bignami M, Brüschweiler B, Ceccatelli S, Cottrill B, Dinovi M, Edler L, Grasl-Kraupp B, Hogstrand C, Hoogenboom LR, Nebbia CS, Oswald IP, Petersen A, Rose M, Roudot A-C, Schwerdtle T, Vleminckx C, Vollmer G, Wallace H, Ruiz Gomes JA and Binaglia M, 2017. Statement on the risks for human health related to the presence of pyrrolizidine alkaloids in honey, tea, herbal infusions and food supplements. EFSA Journal 2017;15(7):4908, 34 pp. https://doi.org/10.2903/j.efsa.2017.4908

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Table of contents

1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

1.1.1. Background

The EFSA Panel on Contaminants in the Food Chain (CONTAM) adopted in 2011 a scientific opinion on pyrrolizidine alkaloids in food and feed.[1]

In this scientific opinion, the CONTAM Panel performed estimates of both acute and chronic exposure to pyrrolizidine alkaloids through honey. Due to lack of data on the presence of pyrrolizidine alkaloids (PAs) in foods other than honey, the CONTAM Panel was not able to quantify dietary exposure from food other than honey. A number of PAs of particular importance for food and feed were identified and recommended to be included in future monitoring of the presence of PAs feed and food. The CONTAM Panel concluded that 1,2-unsaturated PAs may act as genotoxic carcinogens in humans. Therefore, the CONTAM Panel decided to apply the Margin of Exposure (MOE) approach. A benchmark dose lower confidence limit for a 10% excess cancer risk ($BMDL_{10}$) of 70 μg/kg body weight (bw) per day was calculated as the reference point (RP) for comparison with the estimated dietary exposure. The CONTAM Panel concluded that there is a possible health concern for those toddlers and children who are high consumers of honey.

It was furthermore concluded that, although no occurrence data were available, exposure to PAs from pollen, tea, herbal infusions and herbal dietary supplements could potentially present a risk of both acute and chronic effects in the consumer.

Following the outcome of this scientific opinion from the CONTAM Panel on PAs in food and feed and the availability of new occurrence data on the presence of PAs in food, the Commission requested EFSA for a dietary exposure assessment to PAs in honey, tea, herbal infusions (herbs) and food supplements.

Following this request, EFSA approved on 13 July 2016 a scientific report on the 'Dietary exposure to PAs in the European population'.[2]

Chronic and acute dietary exposure to PAs was estimated in the European population via the consumption of plant-derived foods. This resulted in highest estimates of mean chronic dietary exposure of 34.5–48.4 ng/kg bw per day in 'Toddlers' (LB–UB[3]) and 154–214 ng/kg bw per day in the highly exposed population (LB–UB, also in 'Toddlers'). Following a rather conservative scenario, the highest estimates of acute mean exposure and 95th percentile exposure were calculated for 'Toddlers', with mean exposure up to 311 ng/kg bw per day and 95th percentile exposure up to 821 ng/kg bw per day. Tea and herbal infusions were by far the main average contributors to the total exposure to PAs. Among consumers only, in the adult population, the mean chronic exposure via the consumption of honey ranged between 0.1 and 7.4 ng/kg bw per day (minimum LB–maximum UB), while for high consumers, it was between 0.4 and 18 ng/kg bw per day (minimum LB–maximum UB). In the young population, for the average consumers of honey, estimates were between 0.3 and 27 ng/kg bw per day (minimum LB–maximum UB), and between 0.7 and 31 ng/kg bw per day (minimum LB–maximum UB) among the high consumers. Ad hoc exposure scenarios for food supplements via consumption of pollen-based supplements showed chronic exposure to PAs that ranged between 0.7 and 12 ng/kg bw per day (minimum LB–maximum UB), while acute exposure was between 2.8 and 44 ng/kg bw per day (minimum LB–maximum UB), in both cases among consumers only. Likewise, the consumption of 150 mL infusion of 2 g of selected plant extracts led to exposures to PAs up to 890 ng/kg bw per day (e.g. infusion of Borage).

Following initial discussions on appropriate risk management measures to ensure a high level of human health protection, it was found appropriate to ask EFSA to assess the health risks related to the estimated exposures to PAs from honey, tea, herbal infusions and food supplements. Furthermore the CONTAM Panel is requested to provide an opinion on the PAs of relevance in honey, tea, herbal infusions and food supplements and other feed and food, based on the availability of new occurrence data.

[1] EFSA Panel on Contaminants in the Food Chain (CONTAM); Scientific Opinion on Pyrrolizidine alkaloids in food and feed. EFSA Journal 2011; 9(11):2406, 134 pp. https://doi.org/10.2903/j.efsa.2011.2406. Available online: www.efsa.europa.eu/efsajournal

[2] EFSA (European Food Safety Authority), 2016. Dietary exposure assessment to pyrrolizidine alkaloids in the European population. EFSA Journal 2016;14(8):4572, 50 pp. https://doi.org/10.2903/j.efsa.2016.4572

[3] LB = Lower bound and UB = Upper bound. At the LB, results below the limit of quantification (LOQ) and limit of detection (LOD) were replaced by zero; at the UB, the results below the LOD were replaced by the LOD and those below the LOQ were replaced by the value reported as LOQ.

1.1.2. Terms of Reference

In accordance with Art. 29 (1) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority (EFSA) to assess the human health risks related to the estimated exposures to PAs from honey, tea, herbal infusions and food supplements.

Furthermore the CONTAM Panel is requested to provide an opinion on the PAs of relevance in honey, tea, herbal infusions, food supplements and other feed and food, based on the availability of new occurrence data.

1.2. Interpretation of the Terms of Reference

EFSA received a request from the European Commission to assess the human health risks related to the exposure to PAs from honey, tea, herbal infusions and food supplements estimated in a recent EFSA Technical Report (EFSA, 2016). In addition, an opinion on the PAs of relevance in the aforementioned foods and other feed and food on the basis of the new available occurrence data was requested.

The CONTAM Panel concluded that the EC request can be addressed by a Panel statement including:

- An update of the risk characterisation for human health, considering the new exposure levels estimated by EFSA
- An analysis of the available data sets on the occurrence of PAs in food and feed to recommend a list of PAs of relevance for monitoring in food and feed

The CONTAM Panel concluded that a systematic update of the hazard identification and characterisation performed in the previous opinion (EFSA CONTAM Panel, 2011) was not necessary, also considering the ongoing systematic review under finalisation by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), cfr. Summary Report of the Eightieth JECFA meeting (FAO/WHO, 2015). However, the CONTAM Panel noted that an update of the benchmark dose (BMD) modelling approach applied in the previous opinion is warranted, in view of the new guidance of the EFSA Scientific Committee on the use of BMD in risk assessment (EFSA Scientific Committee, 2017).

1.3. Additional information

Pyrrolizidine alkaloids are a large group of natural toxins synthesised as secondary metabolites by different plant species. Several PAs are known to be highly toxic to humans and animals as a result of their presence in the food chain. In 2011, the CONTAM Panel evaluated the risks to human and animal health related to the presence of PAs in food and feed (EFSA CONTAM Panel, 2011).

PAs can be described as a combination of pyrrolizidine-derived moieties (defined as necine bases) with a pool of different mono- or dicarboxylic acids (defined as necic acids). In particular, the PAs with a double bond in position 1,2 of the pyrrolizidine ring system (1,2-unsaturated PAs) are considered of higher toxicity due to their potential to undergo metabolic activation and form reactive pyrrole species, which can readily react with proteins and form DNA adducts. An in-depth description of the chemistry and biochemistry of PAs is present in the previous CONTAM opinion (EFSA CONTAM Panel, 2011).

The toxicity of PAs in humans is documented in a series of case reports of intoxication following ingestion of PA containing herbal medicines and teas, and outbreak cases including deaths associated with the consumption of grain contaminated with PA containing weeds. Short-term toxicity of PAs includes liver and lung as the main target organs, and in particular it is associated with the onset of hepatic veno-occlusive disease (HVOD). Although most PAs have not been extensively tested in experimental animals or in vitro systems, information on the tested PAs includes hepatotoxicity, developmental toxicity, genotoxicity and carcinogenicity. In particular, 1,2-unsaturated PAs are considered as genotoxic and carcinogenic substances due to their potential to undergo metabolic activation into reactive pyrroles. Based on available data, the International Agency for Research on Cancer (IARC) classified lasiocarpine, monocrotaline and riddelliine as being possibly carcinogenic to humans (category 2B), while other PAs assessed were not classifiable (category 3) due to the limited information available (IARC 1983, 1987, 2002).

1.3.1. Conclusions of the previous opinion of the CONTAM Panel

In 2011, the CONTAM Panel performed a comprehensive risk assessment for the presence of PAs in food and feed considering the information available at the time.

The CONTAM Panel assessed both chronic and acute risks related to the human dietary exposure to PAs. For the chronic effects, the Panel concluded that all 1,2-unsaturated PAs share a common metabolic pathway leading to the formation of genotoxic and carcinogenic reactive pyrroles. The Panel carried out a dose–response analysis for the incidence of liver tumours observed in rodents for two PAs tested in carcinogenicity studies by the National Toxicology Programme (NTP), lasiocarpine and riddelliine. A $BMDL_{10}$ for excess cancer risk of 70 μg/kg bw per day for induction of liver haemangiosarcomas by lasiocarpine in male rats was selected as the RP for the assessment of chronic risks and applied in a MOE approach. In view of the lack of long-term studies for other 1,2-unsaturated PAs, the CONTAM Panel assumed a carcinogenic potency equal to lasiocarpine. This was considered as a conservative approach since lasiocarpine was among the more toxic PAs when comparing intraperitoneal (i.p.) and intravenous (i.v.) acute LD_{50}s, and toxicity of PAs is considered to influence their carcinogenic potency.

The risks related to the possible adverse effect of acute exposure to PAs were assessed considering the available human data. While the CONTAM Panel could not set an acute reference dose (ARfD), the limited information available from human poisoning cases allowed identifying a lowest known dose of approximately 2 mg/kg bw per day associated with acute/short-term effects. This was based on a case of a 6-month-old girl who received a daily dose of approximately 0.8–1.7 mg PA/kg bw for 2 weeks and was diagnosed for HVOD, and a 2-month-old boy who was administered an approximate dose of 3 mg/kg bw for 4 days, with a fatal outcome.

The dietary exposure assessment of the CONTAM Panel 2011 opinion was limited to honey as occurrence data were only available for this food product. Using occurrence data on 14 and 17 PAs from two independent data sets submitted to EFSA, with eight PAs in common between the two data sets, the CONTAM Panel estimated dietary exposure for the consumption of retail honey and for honey purchased locally from a single source. For retail honey, chronic exposure levels up to 37.4 ng/kg bw per day and 9.03 ng/kg bw per day were estimated for children and adults (mean consumption in honey consumers only), respectively. Chronic exposure up to 77.8 ng/kg bw per day and 26 ng/kg bw per day were estimated for 95th percentile consumption in children and adults, respectively. Acute exposure levels up to 254 ng/kg bw and 110 ng/kg bw were estimated considering the 95th PAs concentrations and 95th single day consumption for children and adults, respectively. The exposure estimates calculated in the scenario of honey produced locally from a single source were in general about 50–100% higher than the results of the calculations for retail honey.

In relation to PAs in retail honey, the calculated MOEs for adults (all consumers) ranged from 3,500,000 to 57,000, and from > 7,000,000 to 7,400, at the mean and high (95th percentile) long-term consumption, respectively. For 'toddlers' (all consumers), the MOEs ranged between 7,000,000 and 14,000, and between 7,000,000 and 1,200 for mean and high (95th percentile) long-term consumption, respectively. In the scenarios for consumers only, MOEs for adults were in the range 700,000–7,800 and 230,000–2,700 for mean and 95th percentile consumption, respectively. For 'toddlers', MOEs ranged from 175,000 to 1,900 and from 66,000 to 900 for mean and 95th percentile consumption, respectively. Estimated exposure levels for 'other children' were intermediate between those of 'toddlers' and adults, with corresponding MOEs for all consumers in the ranges of 1,800,000–25,000 and > 7,000,000–3,900 at mean and 95th percentile consumption, respectively. Overall, the Panel concluded that there was a possible health concern for those toddlers and children who are high consumers of honey. Estimates of acute dietary exposure to PAs in honey were four orders of magnitude lower than the lowest known PA dose associated with acute/short-term toxicity in humans, indicating no risk of PA acute toxicity related to consumption of honey. The Panel noted that much higher exposure levels to PAs could result from pollen and herbal dietary supplements than dietary exposure from honey, but data were not available for the CONTAM Panel to perform exposure assessments or risk characterisation for these sources.

For the risk to animal health related to the presence of PAs in feed, no quantitative risk assessment was possible in view of the limited data on occurrence and toxicity of PAs in livestock and domestic animals. Exposure to PAs may occur via the consumption of forage and roughage, or herbs and herbal mixtures contaminated with PA producing plants (e.g. Senecioneae and Boraginaceae spp.). All animal species were considered sensitive to the toxic effects of PAs, with small ruminants and rabbits being more resistant than other species. Overall, the CONTAM Panel concluded that the risk of PA poisoning in the European Union (EU) appears to be low and most poisoning cases reported have been due to accidental exposure.

Finally, the CONTAM Panel identified also PAs of particular importance for food and feed, considering the prominent alkaloids present in the main known PA containing plants. This list was

subsequently taken forward by the European Commission in a recommendation for monitoring PAs in food (SCOFCAH, 2014), although it was noted at the time that analytical standards were available only for some of the PAs listed in EFSA opinion.

1.3.2. Conclusions of the EFSA scientific report on exposure assessment to PAs in food

Following a Commission request, EFSA published in August 2016 a scientific report on the dietary exposure to PAs through the consumption of honey, tea, herbal infusions (herbs) and food supplements (EFSA, 2016).

The scientific report considered the 28 PAs provisionally selected by the European Commission, based on the EFSA opinion (EFSA CONTAM Panel, 2011) and two reports, one EFSA external Scientific report (Mulder et al., 2015) and the other produced by the German Federal Institute for Risk Assessment (BfR, 2013). Initially, 274,632 analytical results were available for the exposure estimations; the concentration of PAs in each food sample was estimated adding up all the individual levels of PAs analysed. For tea and herbal infusions, samples with a minimum of 17 and a maximum of 28 analysed PAs were selected to estimate dietary exposure, while for honey, the number of PAs per sample in the final data set varied between 8 and 19.

Retail honey contained PA concentrations of 14.5–27.5 μg/kg (lower bound–upper bound (LB–UB)). The final data set of tea and herbal infusions contained samples of, among others, 'Tea and herbs for infusions, unspecified' (n = 1,002), 'Black tea, infusion' (n = 339), 'Green tea, infusion' (n = 310), 'Camomile flowers' (n = 256), Peppermint (n = 196) and 'Rooibos' (n = 167). The highest average concentrations of PAs (expressed as consumed) were found in the samples of rooibos (LB = 4.1 μg/L) and peppermint (LB = 3.5 μg g/L). Concentrations of PAs in black tea were twice as high as reported for green tea (LB = 1.6 μg/L and LB = 0.8 μg/L, respectively). Certain food supplements contained very high levels of PAs. Average PA concentrations of 235–253 μg/kg (LB–UB) were reported for pollen-based supplements. Much higher concentrations were reported for some plant extracts consumed as infusions such as borage (*Borago officinalis*) with levels up to 31,101 μg/kg or for comfrey (*Symphytum officinale*) (up to 29,694 μg/kg), both concentrations expressed in the dry product. Some supplements containing plant material and sold as capsules/tablets to be directly ingested possessed the highest levels of PAs (hemp-agrimony (*Eupatorium cannabinum*) up to 2,400 mg/kg).

In order to cover the whole range of concentrations of PAs reported for tea and herbal infusions, the estimation of dietary exposure to PAs considered two different scenarios. Together with the other food commodities, a first scenario considered the samples of tea and herbal infusions submitted by national authorities and those collected through an EFSA Article 36 grant (Scenario A), while a second scenario assessed exposure based on samples of tea and herbal infusions submitted by Tea & Herbal Infusions Europe (THIE) (Scenario B).

In the Scenario A, the highest estimates of mean chronic dietary exposure were rather similar in both the youngest age classes ('Infants' and 'Toddlers') and the oldest age classes ('Elderly', 'Very elderly'). In 'Toddlers' the maximum exposure estimate was 34.5–48.4 ng/kg bw per day (LB–UB) while for 'Very elderly' was 31.1–41.8 ng/kg bw per day (LB–UB). In the highly exposed population (95th percentile), the highest estimates were 153.8–214.0 ng/kg bw per day and 87.7–127.2 ng/kg bw per day (LB–UB) in 'Toddlers' and 'Elderly'–'Very Elderly', respectively.

In Scenario B, the estimates of chronic exposure were lower as compared to the previous scenario. Overall, in 'Infants' and 'Toddlers', the main average contributors were either 'Tea, unspecified' or 'Tea and herbs for infusions, unspecified'. In the adult population, the main contributor to the exposure to PAs was tea; either reported as 'Tea, unspecified' or as 'Black tea, infusion'.

Considering the relatively high levels of PAs in honey and its possible regular consumption by particular subgroups of the population, an ad hoc exposure scenario was applied to estimate the exposure amongst consumers only. In the adult population, the mean chronic exposure via the consumption of honey, among consumers only, ranged between 0.1 and 7.4 ng/kg bw per day (minimum LB–maximum UB), while for high consumers (95th percentile exposure) it was between 9.3 and 17.6 ng/kg bw per day (minimum LB–maximum UB). In the young population, for the average consumers, estimates ranged between 0.3 and 27.0 ng/kg bw per day (minimum LB–maximum UB), and between 0.7 and 31.1 ng/kg bw per day (minimum LB–maximum UB) among the high consumers. Although based on very limited number of eating occasions (n = 32), chronic exposure to PAs via the

consumption of pollen-based supplements was also estimated and ranged between 0.7 and 11.5 ng/kg bw per day among consumers only (minimum LB–maximum UB).

Acute dietary exposure to PAs was estimated following a conservative approach considering the presence of high contamination levels in all the different food commodities combined with the total daily consumption for each corresponding food (consuming days only). The highest estimates of acute mean and high (95th percentile) exposure were calculated for 'Toddlers', being up to 311 ng/kg bw per day and up to 821 ng/kg bw per day, respectively. Likewise, the consumption of 150 mL infusion of 2 g of certain plant extracts with relatively high PA levels can lead to exposure to PAs up to 890 ng/kg bw per day as estimated for one infusion of borage (*B. officinalis*). For pollen-based supplements, the acute exposure was between 2.8 and 43.9 ng/kg bw per day (minimum LB–maximum UB), among consumers only.

On estimating dietary exposure to PAs, the UB levels were highly influenced by the sensitivity of the analytical methods and the large proportion of left-censored data. This was particular evident in the Scenario B, where 93% of the analytical data were left-censored, with almost 60% of the samples of tea and herbal infusions with not a single PA quantified. Based on the current sensitivity of the reported analytical methods for the 28 PAs, the lowest UB concentration that can be achieved for tea and herbal infusions is 33.5 μg/kg (0.45 μg/L). This would correspond to mean chronic exposure UB levels (across age groups) up to 3.9–13.5 ng/kg bw per day, and up to 9.5–18.2 ng/kg bw per day among the highly exposed consumers, depending on the tea and herbal infusion consumed. For honey, the lowest UB concentration that could be reported with the eight selected PAs all at levels below the limit of quantification (LOQ) would be 3 μg/kg. This would lead to mean chronic exposure estimations up to 2.9 ng/kg bw per day, and up to 3.4 ng/kg bw per day among the highly exposed consumers.

In addition to continue ongoing efforts to collect analytical data on the occurrence of PAs in relevant foods, there is a need to develop more sensitive analytical methods allowing the reduction in UB levels, and define performance criteria for the analysis of the most relevant PAs in food.

2. Assessment

2.1. Updated dose–response analysis

The CONTAM Panel agreed that an update of the dose response analysis performed for the chronic effects of PAs in the previous opinion is warranted in view of the updated guidance of the EFSA Scientific Committee on the use of benchmark modelling in risk assessment (EFSA Scientific Committee, 2017).

The CONTAM Panel reviewed the dose–response analysis carried out in 2011, briefly described in Section 1.3.1 of this statement and applied the BMD model averaging approach on the data sets on the incidence of liver haemangiosarcoma in male and female rats exposed to lasiocarpine (NTP, 1978) and riddelliine (NTP, 2003). When analysing the data sets, the CONTAM Panel noted that weaknesses are present in both studies in relation to the application of the BMD approach.

The NTP (1978) study on lasiocarpine reports that 24 rats/sex were tested in each treatment group, a relatively low number of animals considering the population size currently recommended for long-term studies in widely accepted test guideline documents. In addition, high mortality was observed at an early stage of the exposure period in both males and females exposed to the highest tested dose (1.5 mg/kg bw per day), and to a lesser extent the mid tested dose (0.75 mg/kg bw per day). In particular, in males an increased mortality started after week 60 and no rats in the high-dose group survived beyond week 88. Mortality affected more severely the study in female rats, with all animals in the high dose group dying approximately between week 30 and week 68. The impact of early mortality on the incidence of liver haemangiosarcoma was evident in female rats and hindered the possibility to perform BMD analysis on that data set. The Panel noted that early mortality could have also affected the likelihood of observing tumours in males exposed to 1.5 mg lasiocarpine/kg bw per day. Finally, the CONTAM Panel noted that the data set has limitations for the performance of BMD modelling, since all the three tested doses were associated with an increased incidence in liver haemangiosarcoma higher than the default benchmark response (BMR) of 10%.

The study on riddelliine was conducted with an adequate number of animals per dose group, following a tailored study design with six female groups (control and five riddelliine doses) and only two male groups (control and high dose). Also, in this case, early mortality was observed at the top dose (0.714 mg/kg bw per day), however, compared to lasiocarpine, a higher incidence of liver

haemangiosarcoma was observed in both sexes exposed to this high dose (76% and 86% for female and male rats, respectively), suggesting a low impact of the early mortality in the observed dose–response relationship. Even though the study design was particularly suitable for the performance of BMD modelling, the data set on the incidence of liver haemangiosarcoma in female rats was considered by the CONTAM Panel to have limitations as only the highest tested dose induced a statistically significant increase in tumour incidence. No tumour incidence was observed in the control group and in the lower three doses ranging from 0.007 to 0.071 mg/kg bw per day. The increased incidence in liver haemangiosarcoma observed at 0.236 mg/kg bw per day (3 female rats out of 50), although not achieving statistical significance, was considered of biological significance in view of the low spontaneous incidence of this type of tumour in rats (Zwicker et al., 1995).

The BMD modelling of the incidence of liver haemangiosarcoma in male rats exposed to lasiocarpine and in female rats exposed to riddelliine led to BMD_{10} confidence intervals (CIs) ($BMDL_{10}$–$BMDU_{10}$) of 8–343 and 237–548 μg/kg bw per day, respectively, based on model averaging.

Applying model averaging, the BMD_{10} CI for lasiocarpine was affected by a high degree of uncertainty, with a $BMDU_{10}$ to $BMDL_{10}$ ratio of about 40-folds and $BMDL_{10}$–$BMDU_{10}$ intervals below the tested dose range for all the accepted individual models. On the other hand, the BMD modelling for riddelliine using model averaging resulted in a narrower $BMDL_{10}$–$BMDU_{10}$ interval, fully included within the two higher tested doses (equivalent to 237–714 μg/kg bw per day), despite the relatively high uncertainty related to the poor information on the dose response relationship of the study.

Despite the marked difference between the $BMDL_{10}$ for lasiocarpine and riddelliine, mainly due to the aforementioned limitations of the two data sets, a partial overlap of the $BMDL_{10}$–$BMDU_{10}$ CIs calculated using model averaging was observed, suggesting that the two substances could have similar carcinogenic potency. This was more evident when a BMR falling within the tested dose ranges for both substances, such as 30%, was selected. BMD_{30} of 491 and 435 μg/kg bw per day were calculated for lasiocarpine and riddelliine, respectively, using model averaging. The respective $BMDL_{30}$–$BMDU_{30}$ intervals were 211–811 μg/kg bw per day for lasiocarpine and 373–622 μg/kg bw per day for riddelliine. Overall, this additional modelling supported the assumption that the two PAs can be considered of similar carcinogenic potency.

In conclusion, the CONTAM Panel selected the $BMDL_{10}$ of 237 μg/kg bw per day, derived for the incidence of liver haemangiosarcoma in female rats exposed to riddelliine as RP for the chronic risk assessment of PAs. Considering the general degree of uncertainty related to the available studies used for the dose response analysis and the fact that both riddelliine and lasiocarpine are classified among the most potent PAs, the CONTAM Panel concluded that the change in the RP maintains the conservative nature of the previous risk assessment.

The full details of the BMD modelling are given in Appendices A and B.

2.2. Updated risk characterisation

The CONTAM Panel considered that the recent report on dietary exposure assessment to PAs in the European population (EFSA, 2016), and the updated RP for the assessment of carcinogenicity warranted the update of the conclusions on the risks to human health of the previous scientific opinion.

Chronic risks

With regard to the chronic exposure, the CONTAM Panel applied an MOE approach considering the different chronic exposure scenarios presented in the latest exposure assessment, using the chronic RP of 237 μg/kg bw per day for the sum of 1,2-unsaturated PAs assuming equal potency. The EFSA Scientific Committee concluded that, for substances that are both genotoxic and carcinogenic, an MOE of 10,000 or higher, based on a $BMDL_{10}$ from an animal study, and taking into account overall uncertainties in the interpretation, would be of low concern from a public health point of view (EFSA, 2005).

Considering the all consumers scenario using the MS and Art 36 occurrence data sets (Scenario A described in Section 1.3.2), the Panel calculated MOEs ranging from > 10,000,000 to about 4,900 (min LB–max UB across dietary surveys and age classes) for the mean exposure in the younger age classes (infants–adolescents) and from > 1,000,000 to 5,700 (min LB–max UB across dietary surveys and age classes) for adults, as shown in Table 1.

The CONTAM Panel noted that MOEs calculated for all age groups when considering the maximum LB exposure levels are similar to respective MOEs at the maximum UB, indicating that the differences

in consumption data present in the various surveys, rather than the analytical uncertainties in the occurrence data, are mainly responsible of the high variability observed in the minimum LB–maximum UB MOEs.

When considering the 95th percentile exposure levels calculated in Scenario A, MOEs below 10,000 were calculated for all age groups both at the maximum LB and maximum UB. For the younger age classes MOEs ranged from > 10,000,000 to about 1,100 (min LB–max UB across dietary surveys and age classes), and for adults from > 200,000 to about 1,900 (min LB–max UB across dietary surveys and age classes). The median LB–UB 95th percentile ranged from about 16,200 (median LB in 'adolescents') to about 4,200 (median UB in 'toddlers') (see Table 1).

Table 1: Exposure levels calculated in the EFSA report on dietary exposure assessment to pyrrolizidine alkaloids (PAs) (EFSA, 2016), considering data submitted by EU Member States and from an Article 36 Grant project (Mulder et al., 2015) and related Margin of Exposure (MOEs) using the Reference Point of 237 μg/kg bw per day for the sum of all 1,2-unsaturated PAs

Age class		Mean dietary exposure (ng/kg bw per day)						MOEs Mean dietary exposure					
		Lower bound[a]			Upper bound[a]			Lower bound			Upper bound		
	N	Min	Median	Max	Min	Median	Max	Min	Median	Max	Min	Median	Max
Infants	6	0	4.1	30.2	0	5.9	42.8	> 1,000,000	57,805	7,848	> 1,000,000	40,169	5,537
Toddlers	10	0	3.2	34.5	0	5.2	48.4	> 1,000,000	74,063	6,870	> 1,000,000	45,577	4,897
Other children	18	0.7	4.2	24.1	1.2	6.4	34.3	338,571	56,429	9,834	197,500	37,031	6,910
Adolescents	17	0.3	3.7	18.4	0.6	5.7	26.1	790,000	64,054	12,880	395,000	41,579	9,080
Adults	17	0.2	6.7	21.3	0.4	10.6	28.8	1,185,000	35,373	11,127	592,500	22,358	8,229
Elderly	14	3.0	8.1	29.5	4.3	12.4	39.9	79,000	29,259	8,034	55,116	19,113	5,940
Very elderly	12	3.9	9.2	31.1	5.7	13.9	41.8	60,769	25,761	7,621	41,579	17,050	5,670

Age class		95th percentile dietary exposure[b] (ng/kg bw per day)						MOEs 95th percentile dietary exposure					
		Lower bound[a]			Upper bound[a]			Lower bound			Upper bound		
	N	Min	Median	Max	Min	Median	Max	Min	Median	Max	Min	Median	Max
Infants	5	0	_[c]	133.6	0	_[c]	185.2	> 10,000,000		1,774	> 10,000,000		1,280
Toddlers	7	0	42.8	153.8	0	57.1	214	> 10,000,000	5,537	1,541	> 10,000,000	4,151	1,107
Other children	18	3.3	21.2	90.5	6.3	32.5	125.6	71,818	11,179	2,619	37,619	7,292	1,887
Adolescents	17	0.8	14.6	68.4	2.4	24.6	95.1	296,250	16,233	3,465	98,750	9,634	2,492
Adults	17	1.1	30.1	85.7	2.0	42.9	120.0	215,455	7,874	2,765	118,500	5,524	1,975
Elderly	14	15.3	33.8	87.7	21.4	52.7	123.3	15,490	7,012	2,702	11,075	4,497	1,922
Very elderly	9	15.9	30.8	86.7	22.9	42.8	127.2	14,906	7,695	2,734	10,349	5,537	1,863

bw: body weight.
(a): Estimates were rounded to one decimal figure.
(b): The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011). Those estimates were not included in this table.
(c): A minimum number of six dietary surveys is required to estimate a statistically robust median (EFSA, 2011).

The second all consumers chronic scenario was run using the occurrence data set for tea and herbal infusion submitted by THIE (Scenario B in Section 1.3.2). This led to consistently lower exposure estimates compared to the previous scenario, and consequently to higher MOEs. This is reflected in particular in the mean exposure scenario, in which MOEs calculated using maximum UB exposure levels were slightly below 10,000 for 'infants' and 'toddlers' (approximately around 8,000–9,000), higher than 10,000 for 'adolescents' and 'adults' (13,100 and 10,500, respectively) and below 10,000 for 'elderly' and 'very elderly' (7,500 and 7,100, respectively). Maximum UB MOEs calculated for the 95th percentile consumption were consistently below 10,000 for all age groups (ranging approximately from 1,800 to 3,700). A greater difference was observed for MOEs calculated using LB exposure estimates, reflecting the possible limitations of this data set identified in the dietary exposure assessment report, including a lower number of analysed PAs in some samples and a lower analytical sensitivity (EFSA, 2016) (see Appendix C, Table C.1).

Finally the chronic exposure was estimated for consumers only (see Appendix C, Table C.2), considering different types of teas, herbal infusions and honey. In particular, for Scenario A, minimum LB–maximum UB MOEs calculated for the means for only consumers of unspecified herbs and infusions ranged from > 1,000,000 to 4,300 and from > 1,000,000 to 1,000 for the adult and young population, respectively. The 95th percentile MOEs ranged from 395,000 to 1,500 and from 43,000 to 770 for the adult and young population, respectively. When looking at specific types of infusions, lower MOEs were calculated for the consumption of camomile flowers in particular in the young population (minimum LB–maximum UB approximately at 28,200–5,300 for mean consumption, 95th percentile not calculated), and for rooibos leaves both for adults (ranges of 21,500–5700 and 7,200–2,100 for mean and 95th percentile consumption, respectively) and the young population (range of 17,700–2,900, mean consumption, 95th percentile not calculated). A similar trend to the one reported in the scenarios on all consumers was observed when calculating MOEs for only consumers in Scenario B (data not shown).

MOEs calculated for adult consumers only of retail honey ranged between > 1,000,000 and 32,000 (minimum LB–maximum UB) and between 593,000 and 13,500 for mean and 95th percentile consumption, respectively. For the young population, MOEs ranged from 790,000 to 8,800, and from 339,000 to 7,600 for mean and 95th percentile consumption, respectively.

Regarding the interpretation of the calculated MOEs, the CONTAM Panel noted that a substantial degree of uncertainty remains in relation to the assumption that all 1,2-unsaturated PAs share the same mode of action and have carcinogenic potencies equal to the one selected for the establishment of the RP for neoplastic effects, riddelliine. While it is plausible to assume that following systemic absorption all 1,2-unsaturated PAs will generate the reactive pyrrole species likely responsible of the adverse effects, a large variability in toxicokinetic and toxicodynamic can be also expected in view of the large structural diversity in this group of substances, which could result in a marked variability in the carcinogenic potency of the individual PAs. In a recent work, Merz and Schrenk (2016) proposed provisional potency factors for a series of 1,2-unsaturated PAs, based on available data on i.p. and i.v. acute LD_{50}s in rat and mouse, genotoxic potency in *Drosophila melanogaster*, and *in vitro* cytotoxicity data in a model of chicken hepatocytes. From the analysis of this composite data set, the authors proposed a rationale to differentiate carcinogenic potency of 1,2-unsaturated PAs, based on the structure and stereochemistry of their necic acid moieties. In particular, cyclic diesters and open-chained diesters with 7*S* configuration (e.g. lasiocarpine, riddelliine or senecionine) were assigned a relative potency factor (RPF) of 1; monoesters with 7*S* configuration (e.g. heliotrine) were assigned RPF of 0.3; and finally open-chained diesters with 7*R* configuration (e.g. echimidine) and 7*R*-monoesters (e.g. intermedine or lycopsamine) were assigned RPF values of 0.1 and 0.01, respectively. In a more recent approach, Chen et al. (2017) proposed to derive RPFs for a series of PAs for which information on tumour incidence following exposure in rats is available. Beside the two PAs with available oral carcinogenicity studies (lasiocarpine and riddelliine), this series includes monocrotaline, clivorine, senkirkine and symphytine, for which limited information is available on their carcinogenic potency. Namely, these substances were studied in tests with design limitations (only one dose group and a control group, limited number of animals and non-standard exposure regime, including shorter durations and treatment frequencies). In addition, only the study on clivorine was carried out using the oral route of exposure, whereas i.p. injection was used in the studies on senkirkine and symphytine, and s.c. injection for monocrotaline. Chen et al. (2017) derived RPFs by estimating the doses associated with an increase of 10% in tumour incidence (T10) for monocrotaline, clivorine, senkirkine and symphytine and comparing them with the RP derived by EFSA in 2011 for lasiocarpine. In the case of riddelliine, the lowest $BMDL_{10}$ calculated by EFSA in 2011 was selected for

the comparison. This resulted in RPFs of 1, 0.39, 0.05, 0.23, 0.03 and 0.02 for lasiocarpine, riddelliine, monocrotaline, clivorine, senkirkine and symphytine, respectively. Finally, in a comparative 28-day oral toxicity study recently performed by Dalefield et al. (2016) on echimidine and lasiocarpine, Wistar rats (10/sex per dose) were exposed to either one of these two PAs at doses of 0.6, 1.2 or 2.5 mg/kg bw, including a common negative control group. A significant decrease in body weight gain was observed in male and female rats treated with lasiocarpine at $\geq$ 1.2 and 2.5 mg/kg bw per day, respectively, while no effects on body weight gain were observed in the groups treated with echimidine. No other adverse effects were observed for the two substances. The CONTAM Panel concluded that, due to the limitations in the analysed data set and the provisional nature of the semi-quantitative approach proposed by Merz and Schrenk (2016), it is not adequate to use the derived RPFs for the cumulative risk assessment of PAs in food. Similarly, the approach proposed by Chen et al. (2017) has also important limitations and its use is not considered adequate for the risk assessment of PAs. However, altogether these publications suggest that several of the PAs mainly contributing to the dietary exposure levels calculated in the EFSA report (2016) could be of substantially lower potency than riddelliine or lasiocarpine. As already discussed in the 2011 opinion, The CONTAM Panel therefore confirmed the conservative nature of the RP based on potent PAs such as riddelliine or lasiocarpine for the cumulative risk assessment of PAs in food.

The CONTAM Panel concluded that the MOEs calculated for all consumers in the mean and high (95th percentile) consumption scenarios indicate a possible concern for human health. In particular a concern was expressed for frequent and high consumers of teas or herbal infusions.

Acute risks

As described in Section 1.3.1, an approximate lowest known dose of 2 mg PA/kg bw per day associated with acute/short-term toxicity in humans was used by the CONTAM Panel for the assessment of acute risks, based on information from human cases indicating short-term toxicity following exposure in the range 1–3 mg PA/kg bw per day for periods ranging from 4 days up to 2 weeks.

In the 2016 EFSA report, acute dietary exposure to PAs was estimated considering the presence of high contamination levels in all the different food commodities, combined with the total daily consumption for each corresponding food. This conservative approach resulted in acute exposure levels ranging from approximately 1 to 300 ng/kg bw per day and from 6 to 170 ng/kg bw per day for mean consumers in the younger age classes (infants–adolescents) and adults, respectively. Exposure for the 95th percentile consumption levels was well below 1 μg/kg bw per day in all age classes. In view of the margin of more than three orders of magnitude between the estimated exposure levels and the lowest known dose range of 1–3 mg PA/kg bw per day at which human acute/short-term toxicity has been reported, the CONTAM Panel concluded that there is a low risk related to acute dietary exposure to PAs through the consumption of teas, herbal infusions and honey.

In specific scenarios, the acute (or short-term, assuming daily consumption of the same food supplement batch for few days/weeks) exposure to PAs related to the consumption of food supplements was estimated. In the 2016 dietary exposure report of EFSA, a wide range of PA concentrations was reported for herbal food supplements, reaching total PA levels of more than 2 g/kg in some samples. Acute/short-term exposure was estimated for plant extracts intended to be consumed following infusion (by assuming the same dilution factor used for teas and herbal infusions) or to be ingested as capsules/tablets. A single consumption occasion of a *B. officinalis* infusion led to an estimated acute/short-term exposure of 890 ng/kg bw per day. In another scenario, ingestion of one tablet/capsule of boneset (*Eupatorium perfoliatum*) or hemp-agrimony (*E. cannabinum*) corresponded to estimated acute/short-term exposure levels of about 800–1,800 μg/kg bw per day, respectively. Acute/short-term exposure through the consumption of pollen-based supplements showed much lower exposure estimates in the range of 3–44 ng/kg bw per day.

The CONTAM Panel concluded that the consumption of herbal food supplements based on PA-producing plants could reach acute/short-term exposure levels in the range of doses associated with severe acute/short-term effects in humans. This is supported by a series of human cases of intoxication following the consumption of herbal remedies derived from PA-producing plants (EFSA CONTAM Panel, 2011). In view of the uncertainty on the possible toxicity levels of PAs in humans and of the severity of the effects, the CONTAM Panel concluded that exposure levels less than 100 times lower than the aforementioned dose range of 1–3 mg PA/kg bw per day may be associated with the risk of acute/short-term effects.

Consumption of pollen-based supplements is not considered to pose acute risks to human health.

2.3. Recommended PAs for monitoring in food and feed

2.3.1. Food

Together with the estimation of the dietary exposure to PAs in the European population, the 2016 EFSA scientific report carried out an exhaustive evaluation of the available occurrence data in diverse food commodities, including the contribution of each PAs to the total contamination levels in the samples (EFSA, 2016).

Considering the final data set of tea and herbal infusions, the main average contributors to the total PA concentration in green tea were senecionine-*N*-oxide (19%), retrorsine-*N*-oxide (18%), and intermedine and lycopsamine, both with 16% contribution. In black tea, the main contributors, on average, were intermedine-*N*-oxide (31%), intermedine (20%), lycopsamine (20%) and retrorsine-*N*-oxide (15%); in camomile, senecionine-*N*-oxide (28%), intermedine (22%), senecionine and lycopsamine (both 10%); in peppermint, seneciphylline-*N*-oxide (28%), senecionine-*N*-oxide (25%), retrorsine-*N*-oxide (13%) and seneciphylline (11%); and in rooibos, senecionine-*N*-oxide (57%), retrorsine-*N*-oxide (19%) and senecionine (16%).

Overall, among the samples of tea and herbal infusions, the main contributors to the total PA concentration were, on average: lycopsamine, intermedine, intermedine-*N*-oxide, senecionine, senecionine-*N*-oxide, seneciphylline, seneciphylline-*N*-oxide and retrorsine-*N*-oxide. In black tea, these eight PAs represented, on average, 95% of the total PA concentration, 92% in samples of rooibos, 90% in samples of camomile, 83% in samples of peppermint and 78% in green tea.

Among the samples of retail honey, the main contributors to the total PA concentration in each sample were, on average, echimidine (44%) and lycopsamine (37%). Similar main contributors had been already described for the 1,324 samples available in the 2011 CONTAM opinion that were also part of the 1,966 samples used in the 2016 EFSA scientific report.

For food supplements (plant extracts and pollen-based supplements) overall, the highest average contributions to the total PA levels came from lycopsamine, intermedine and their *N*-oxides. An exception was the samples of coltsfoot, where 80–90% of the total concentration of PAs came from senkirkine.

Together with their occurrence in the different food commodities, other criteria such as the toxicology and chromatographic separation were also considered when selecting a set of PAs to be monitored.

From an analytical point of view, the analysis of certain PA isomers such as intermedine/lycopsamine or senecionine/senecivernine as well as their *N*-oxide derivatives present certain difficulties. It is reported that by using high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS), the most habitual analytical method for the analysis of PAs, a baseline chromatographic separation is not always achieved for these PAs (Crews, 2013). In addition, they have the same molecular weight and cannot be distinguished by mass spectrometry. Due to this fact, it seems that an accurate quantification of the individual PAs is not always possible. For the pair intermedine/lycopsamine also co-elution of indicine could happen (Mulder et al., 2015). An identical situation is observed for indicine-*N*-oxide and the *N*-oxide derivatives of intermedine/lycopsamine.

Another issue to be considered is that the ratio between the two forms usually present, the PA-*N*-oxide (PANO) and the free tertiary base, strongly depends of many factors among them the sample preparation and extraction conditions (EFSA CONTAM Panel, 2011). Therefore, a general recommendation is to analyse both forms regardless of the PAs selected.

Other PAs that should be monitored, although not relevant in terms to their contribution to the total occurrence in the current data set, are lasiocarpine and senkirkine. Lasiocarpine is among the most toxic of the PAs that have been tested, and the $BMDL_{10}$ for induction of liver haemangiosarcomas in male rats was used as RP in the previous EFSA opinion (EFSA CONTAM Panel, 2011). In addition, and although lasiocarpine was only quantified in less than 5% of the samples analysed, in certain food categories in particular in 'Tea for infants and young children', this PA represented on average 42% of the total concentration among the samples where it was analysed. Regarding, senkirkine, its average contribution to the occurrence levels in the food commodities (honey and tea/herbal infusions) was negligible (e.g. 0.9% in honey and 1.7% in green tea). However, senkirkine can be of particular importance in certain plant extracts, such as *Tussilago farfara* (coltsfoot), samples with reported PA levels above 400 μg/L and with this PA contributing to 80–90% of the total concentration.

Based on this information, the CONTAM Panel proposed a set of 17 PAs to be monitored in food: intermedine/lycopsamine, intermedine-*N*-oxide/lycopsamine-*N*-oxide, senecionine/senecivernine, senecionine-*N*-oxide/senecivernine-*N*-oxide, seneciphylline, seneciphylline-*N*-oxide, retrorsine, retrorsine-*N*-oxide, echimidine, echimidine-*N*-oxide, lasiocarpine, lasiocarpine-*N*-oxide and senkirkine. When considering this list, it should be taken into account that diverse PAs may co-elute with some of the PAs included. This is the case for instance of indicine and indicine-*N*-oxide that, however, are not relevant PAs in food. Under certain analytical conditions, these compounds may not be completely separate from the pair intermedine/lycopsamine and their respective *N*-oxides.

In addition to the proposed 17 PAs, recent analyses of tea samples (personal communication, Dr. Patrick Mulder, RIKILT) seem to indicate that other PAs could also have a relevant contribution to the levels of PAs in different foods. This refers mainly to integerrimine and echinatine together with their *N*-oxides which are not always chromatographically separated to baseline from the pairs senecionine-*N*-oxide/senecivernine-*N*-oxide and intermedine-*N*-oxide/lycopsamine-*N*-oxide, respectively. While echinatine is a structural isomer of lycopsamine and intermedine being a relevant PA in *Eupatorium* and *Cynoglossum* species, integerrimine has been described in *T. farfara* and *Senecio vulgaris* plants (El-Shazly and Wink, 2014; Nedelcheva et al., 2015).

Therefore, and based on standard availability, PAs other than those included in the proposed list of 17 PAs should be also monitored to better understand the occurrence of PAs in food.

Following the approach used in the 2016 EFSA scientific report (EFSA, 2016), an hypothetical scenario was built to assess what would be the dietary exposure in the European population if all results were below LOQ, based on the performance of current analytical methods for PAs (as provided in Table 12 of the 2016 EFSA scientific report). Estimates of dietary exposure to PAs were calculated assuming that all 17 PAs from the proposed list were below the LOQ, summing the 17 LOQs and combining the resulting value with the consumption of different food commodities (consumers only). Among the young population ('Infants', 'Toddlers' and 'Other children'), the maximum mean dietary exposure was estimated for 'Tea and herbs for infusions, unspecified' being 7.5 ng/kg bw per day, and a maximum 95th exposure of 10.1 ng/kg bw per day in the same food commodity. In the adult population ('Adults', 'Elderly' and 'Very elderly') highest mean exposure was estimated with the consumption of 'Tea and herbs for infusions, unspecified' being 5.2 ng/kg bw per day, while the maximum 95th exposure was estimated via the consumption of 'Tea unspecified, decaffeinated' to be 5.3 ng/kg bw per day. More details for the different food commodities and the range of chronic exposure estimates across the different dietary surveys is given in Appendix D. It can be noted that the application of the MOE approach to the exposure estimates reported in Appendix D and using the chronic RP of 237 μg/kg bw per day for the sum of 1,2-unsaturated PAs assuming equal potency, would result in MOEs above 10,000.

2.3.2. Feed

A total of 29,739 analytical results were available on different PAs, for a total of 524 samples. Samples were collected between 2006 and 2016, with 438 samples collected in the Netherlands and 86 in the Czech Republic. As compared to the situation at the moment of the publication of the 2011 CONTAM opinion, only few more samples (173) were available, 87 collected in the Netherlands and 86 in the Czech Republic. Samples collected in the Czech Republic were analysed for either four PAs (retrorsine, seneciphylline, senecionine and senkirkine, 37 samples) or five PAs (same PAs as before + monocrotaline, 49 samples).

All the samples collected in the Netherlands were analysed for 67 PAs, including 26 out of the 28 PAs provisionally selected by the European Commission (individual results for intermedine and its *N*-oxide were not reported). Following a clarification request to the data provider, it was confirmed that the analytical method was not able, at that time, to distinguish between intermedine/lycopsamine and intermedine-*N*-oxide/lycopsamine-*N*-oxide so the results were reported as lycopsamine and lycopsamine-*N*-oxide.

All samples from the Czech Republic were left-censored data; **Table 2**, therefore, shows the levels of PAs reported only for the samples collected in the Netherlands. Feed samples were classified according to the Catalogue of feed materials as described in Commission Regulation (EU) No 68/2013[4].

[4] Commission Regulation (EU) No 68/2013 of 16 January 2013 on the Catalogue of feed materials. OJ L 29, 30.1.2013, p. 1–64.

Table 2: Mean values of PAs reported for different types of feed samples collected in the Netherlands

		N	Number of LC	Mean concentration (μg/kg)		
				Lower bound	Middle bound	Upper bound
Cereal grains, their products and by-products	Wheat	1	0	23	171	320
	Maize	4	4	0	151	302
	Millet	4	4	0	151	302
	Oats	1	1	0	151	302
	Rice, broken	3	3	0	151	302
	Sorghum; [Milo]	2	2	0	151	302
Oil seeds, oil fruits, and products derived thereof	Palm kernel expeller	4	4	0	151	302
	Rape seed	4	1	9	159	308
	Toasted soya (beans)	46	37	3	153	303
	Sunflower seed	6	5	5	155	305
	Linseed	11	6	30	177	325
Legume seeds and products derived thereof	Peas	7	6	16	166	315
	Carob, dried	2	1	8	156	305
	Sweet lupins	4	4	0	151	302
Tubers, roots, and products derived thereof	Carrots	1	1	0	151	302
Other seeds and fruits, and products derived thereof	Other seeds and fruits, and products derived thereof	2	1	22	169	316
	Citrus pulp	3	2	12	161	311
Forages and roughage, and products derived thereof	Lucerne, alfalfa	149	18	368	503	637
	Grass, field dried, hay	152	117	174	322	470
Other plants, algae and products derived thereof	Other plants, algae and products derived thereof	32	12	290	435	580

N: Number of samples; LC: left-censored (samples with no PAs quantified).
The concentration in each sample was derived by summing the concentrations reported for each of the 67 PAs analysed.

The list of the 67 PAs analysed in the samples collected in the Netherlands is shown in **Table 3**. The sensitivity of the method (liquid chromatography with tandem mass spectrometry (LC–MS/MS)) was 4.5 μg/kg for all PAs, expressed as limit of detection (LOD). Out of the 438 feed samples, at least one PA was reported for 209 samples.

Regarding the 28 PAs that belong to the list provisionally selected by the European Commission, they were quantified at 803 occasions (7%), with seneciphylline (n = 129) and seneciphylline-*N*-oxide (n = 103), reported the most. For the 41 PAs that are not part of the Commission list, they were quantified in 579 occasions (3%) in 143 different samples. Among those quantified the most often occurring were: integerrimine (n = 66), integerrimine-*N*-oxide (n = 60), spartioidine (n = 60), spartioidine-*N*-oxide (n = 47), iso-acetylechimidine (n = 40), iso-echimidine (n = 41), riddelliine (n = 39) and riddelliine-*N*-oxide (n = 35).

Table 3: List of PAs analysed in samples collected in the Netherlands

Acetylheliosupine	Acetylheliosupine-*N*-oxide	Acetylechinatine	Acetylechinatine-*N*-oxide	Acetylerucifoline	Acetylerucifoline-*N*-oxide
Acetylseneciphylline	Acetylseneciphylline-*N*-oxide	Acetyllycopsamine	Acetyllycopsamine-*N*-oxide	Acetylechimidine	Acetylechimidine-*N*-oxide
Doronine	Desacetyldoronine	Dehydrojaconine	Echinatine	Echinatine-*N*-oxide	**Echimidine**
Echimidine-*N*-oxide	Echiumine	Echiumine-*N*-oxide	**Europine**	**Europine-*N*-oxide**	**Erucifoline**
Erucifoline-*N*-oxide	Florosenine	Floridanine	**Heliotrine**	**Heliotrine-*N*-oxide**	Heliosupine
Heliosupine-*N*-oxide	Heleurine-*N*-oxide	Integerrimine	Integerrimine-*N*-oxide	**Jacobine**	**Jacobine-*N*-oxide**
Jacoline	Jacoline-*N*-oxide	Jaconine	Jaconine-*N*-oxide	Jacozine	Jacozine-*N*-oxide
Lasiocarpine	**Lasiocarpine-*N*-oxide**	**Monocrotaline**	**Monocrotaline-*N*-oxide**	**Lycopsamine**	**Lycopsamine-*N*-oxide**
Otosenine	Onetine	**Retrorsine**	**Retrorsine-*N*-oxide**	Riddelliine	Riddelliine-*N*-oxide
Senecionine	**Senecionine-*N*-oxide**	**Senecivernine**	**Senecivernine-*N*-oxide**	Spartioidine	**Seneciphylline**
Seneciphylline-*N*-oxide	**Senkirkine**	Spartioidine-*N*-oxide	**Trichodesmine**	**Trichodesmine-*N*-oxide**	Usuramine
Usaramine-*N*-oxide					

Those PAs included among the 28 provisionally selected by the European Commission are in bold (intermedine/lycopsamine and intermedine-*N*-oxide/lycopsamine-*N*-oxide were reported as lycopsamine and lycopsamine-*N*-oxide, respectively, as they were not resolved by the analytical method used).

Some further assessments for the contribution of the different analysed PAs were focused on the two feed categories best represented: '*Lucerne (alfalfa)*' and '*Grass, field dried (hay)*', since they were the only feed groups with a relatively high number of samples quantified (n = 131 and n = 35, respectively). The feed group 'Other plants, algae and products derived thereof' (n = 32) covered a very heterogeneous number of samples, with seventeen different types of plants and six samples reported as 'Herbal mix' without further information. In most of the cases, only one or two samples were available for each type of plant, making any interpretation either on the PA levels or on the profile of PAs reported difficult (see **Table 4**).

Table 4: Samples of 'Other plants, algae and products derived thereof' collected in the Netherlands

		Mean concentration (µg/kg)				
		N	Number of LC	Lower bound	Middle bound	Upper bound
Other plants, algae and products derived thereof	Herbal mix	6	0	353	492	630
	Herbal mix, artichoke	1	0	2,252	2,385	2,517
	Herbal mix, camomile	2	1	35	184	334
	Herbal mix, dandelion	2	1	663	793	924
	Herbal mix, fennel	2	1	1,592	1,732	1,871
	Herbal mix, ginseng	1	0	5	154	302
	Herbal mix, goldenrod	2	0	18	165	312
	Herbal mix, knotweed	1	0	97	241	385
	Herbal mix, leek	1	1	0	151	302
	Herbal mix, marigold	1	1	0	151	302
	Herbal mix, milk thistle	1	0	12	161	309
	Herbal mix, mint	2	2	0	151	302
	Herbal mix, nettle	5	3	16	165	314
	Herbal mix, oregano	1	0	89	235	381
	Herbal mix, parsley	1	1	0	151	302
	Herbal mix, rose hip	1	1	0	151	302
	Herbal mix, rosemary	1	0	5	154	302
	Herbal mix, verbena	1	0	18	164	310

N: Number of samples; LC: left-censored (samples with no PAs quantified).
The concentration in each sample was derived by summing the concentrations reported for each of the 67 PAs analysed.

Grass, hay

In a total of 35 samples among the 152 analysed, at least one PA was quantified (23%). In almost half of these samples (17), the PAs from the Commission list represented 100% of the total concentration, while in 10 samples (29%) they represented below 60% of the total. On average, the PAs from the European Commission list represent 78% of the PA levels reported in hay. When looking at the potential contribution of the 17 PAs suggested to be monitored in food, the average contribution in the 35 samples was 69% of the total, in 15 samples representing 100%.

There was one sample where none of the PAs from the Commission list was quantified; the only PAs quantified was acetylerucifoline-*N*-oxide. Overall, the other 41 PAs were identified in total in 88 occasions (18 samples), with no PA standing up among the others in number of occasions reported as quantified (acetylerucifoline, n = 6).

Lucerne, alfalfa

In a total of 131 samples among the 149 analysed, at least one PA was quantified (88%). In 26 samples (20%), the PAs from the Commission list represented 100% of the total concentration, while in only 32 (24%) they represented less than 60% of the total (**Table 5**). On average, the PAs from the Commission list represented 72% of the PA levels reported. In these 32 samples, the most important PAs outside those from the Commission list were acetylheliosupine and heliosupine that represented on average 23% and 16% of the total concentration, respectively.

Concerning the list of 17 PAs suggested to be monitored in food, they represented, on average, 68% of the total PA levels, in 23 of the samples representing 100% and in another 70 samples above 60%.

Overall, the 41 PAs not on the European Commission list were quantified in 449 occasions (118 samples); those that were more often reported as quantified were integerrimine (n = 58), spartioidine (n = 55) and integerrimine-*N*-oxide (n = 52). It is also worth mentioning that riddelliine and riddelliine-*N*-oxide were reported as quantified in 34 and 32 occasions, respectively (**Table 6**).

Table 5: Presence of the PAs from the Commission list in different samples of '*Lucerne, alfalfa*' and '*Grass, field dried (hay)*') quantified for at least for one PA

	Lucerne; (alfalfa)				Grass, field dried, (hay)			
	N[(a)]	Contribution			N[(a)]	Contribution		
		Quantified[(b)]	Average	Max		Quantified[(b)]	Average	Max
Echimidine	131	1	0.1	14.0	35	0	0.0	0.0
Echimidine-*N*-oxide	131	6	1.0	86.0	35	1	0.0	0.1
Europine	131	0	0.0	0.0	35	0	0.0	0.0
Europine-*N*-oxide	131	0	0.0	0.0	35	0	0.0	0.0
Heliotrine	131	0	0.0	0.0	35	0	0.0	0.0
Heliotrine-*N*-oxide	131	0	0.0	0.0	35	0	0.0	0.0
Erucifoline	131	10	0.3	25.7	35	8	2.3	25.7
Erucifoline-*N*-oxide	131	6	0.8	100.0	35	5	2.5	27.2
Jacobine	131	15	1.6	100.0	35	6	1.8	17.1
Jacobine-*N*-oxide	131	4	0.8	100.0	35	3	2.5	77.8
Lasiocarpine	131	0	0.0	0.0	35	0	0.0	0.0
Lasiocarpine-*N*-oxide	131	0	0.0	0.0	35	0	0.0	0.0
Lycopsamine[(c)]	131	29	2.0	38.5	35	8	5.9	67.3
Lycopsamine-*N*-oxide[(c)]	131	10	1.2	100.0	35	8	8.5	100.0
Monocrotaline	131	0	0.0	0.0	35	0	0.0	0.0
Monocrotaline-*N*-oxide	131	0	0.0	0.0	35	0	0.0	0.0
Retrorsine	131	78	7.3	43.9	35	6	1.4	17.5
Retrorsine-*N*-oxide	131	71	7.6	43.2	35	8	4.5	100.0
Senecionine	131	81	7.9	100.0	35	8	2.0	15.8
Seneciphylline	131	107	20.1	100.0	35	19	26.3	100.0
Senecionine-*N*-oxide	131	66	5.2	33.3	35	10	4.7	100.0
Seneciphylline-*N*-oxide	131	84	13.8	100.0	35	14	15.6	100.0
Senecivernine	131	34	0.8	7.8	35	2	0.1	3.5
Senecivernine-*N*-oxide	131	19	0.6	11.1	35	0	0.0	0.0
Senkirkine	131	2	0.8	100.0	35	1	0.0	0.5
Trichodesmine	131	0	0.0	0.0	35	0	0.0	0.0

(a): Number of samples with at least one PA quantified.
(b): Number of times quantified.
(c): Intermedine/lycopsamine and intermedine-*N*-oxide/lycopsamine-*N*-oxide were reported as lycopsamine and lycopsamine-*N*-oxide respectively as they were not resolved by the analytical method used.

Table 6: Presence of PAs other than those from the Commission list in different samples of '*Lucerne, alfalfa*' and '*Grass, field dried (hay)*') quantified for at least for one PA

	Lucerne; (alfalfa)				Grass, field dried, (hay)			
	N[(a)]	Contribution			N[(a)]	Contribution		
		Quantified[(b)]	Average	Max		Quantified[(b)]	Average	Max
Acetyllycopsamine	131	10	2.2	82.3	35	3	1.0	26.0
Acetyllycopsamine-*N*-oxide	131	1	0.0	1.5	35	3	0.4	10.1

	Lucerne; (alfalfa)				Grass, field dried, (hay)			
	N[(a)]	Contribution			N[(a)]	Contribution		
		Quantified[(b)]	Average	Max		Quantified[(b)]	Average	Max
Acetylechimidine	131	0	0.0	0.0	35	0	0.0	0.0
Acetylechimidine-*N*-oxide	131	0	0.0	0.0	35	0	0.0	0.0
Acetylerucifoline	131	4	0.1	5.3	35	6	1.5	40.0
Acetylerucifoline-*N*-oxide	131	0	0.0	0.0	35	4	3.2	100.0
Acetylseneciphylline	131	1	0.2	22.7	35	1	0.1	3.2
Acetylseneciphylline-*N*-oxide	131	0	0.0	0.0	35	1	0.1	5.2
Dehydrojaconine	131	0	0.0	0.0	35	1	0.0	1.2
Desacetyldoronine	131	0	0.0	0.0	35	3	0.3	5.8
Doronine	131	0	0.0	0.0	35	1	0.1	4.8
Echiumine	131	7	0.1	4.7	35	2	0.3	5.5
Echiumine-*N*-oxide	131	2	0.0	2.5	35	3	2.9	65.7
Floridanine	131	0	0.0	0.0	35	1	0.2	7.0
Florosenine	131	1	0.0	0.4	35	2	0.5	9.2
Heleurine-*N*-oxide	131	0	0.0	0.0	35	0	0.0	0.0
Integerrimine	131	58	2.5	13.6	35	5	0.5	5.4
Integerrimine-*N*-oxide	131	52	2.2	20.0	35	4	0.5	8.2
Acetylheliosupine	131	35	6.2	62.7	35	4	2.3	36.0
Acetylheliosupine-*N*-oxide	131	22	1.3	21.5	35	2	0.6	18.4
Acetylechinatine-*N*-oxide	131	0	0.0	0.0	35	2	0.1	2.5
Acetylechinatine	131	2	0.0	2.1	35	2	0.2	5.5
Heliosupine	131	32	4.3	50.0	35	5	1.4	20.0
Heliosupine-*N*-oxide	131	19	1.1	22.2	35	1	0.2	7.8
Echinatine	131	4	0.3	30.6	35	1	0.1	2.5
Echinatine-*N*-oxide	131	1	0.0	0.7	35	0	0.0	0.0
Jacoline	131	0	0.0	0.0	35	1	0.1	1.8
Jacoline-*N*-oxide	131	0	0.0	0.0	35	1	0.0	0.7
Jaconine	131	11	0.3	22.7	35	4	1.0	15.2
Jaconine-*N*-oxide	131	0	0.0	0.0	35	1	0.1	4.2
Jacozine	131	2	0.0	3.4	35	1	0.0	0.2
Jacozine-*N*-oxide	131	0	0.0	0.0	35	1	0.0	0.4
Onetine	131	0	0.0	0.0	35	2	0.2	8.7
Otosenine	131	1	0.0	0.3	35	3	1.1	20.0
Riddelliine	131	34	1.0	12.2	35	5	0.3	5.8
Riddelliine-*N*-oxide	131	32	0.9	19.0	35	2	0.1	1.7
Spartioidine	131	55	2.9	21.4	35	4	0.4	9.8
Spartioidine-*N*-oxide	131	42	2.2	45.2	35	4	2.0	41.7
Trichodesmine-*N*-oxide	131	0	0.0	0.0	35	0	0.0	0.0
Usaramine-*N*-oxide	131	10	0.2	9.5	35	1	0.0	0.4
Usuramine	131	12	0.1	2.1	35	1	0.0	0.0

(a): Number of samples with at least one PA quantified.
(b): Number of times quantified.

Further attention was also paid in highly contaminated samples to the contribution of the PAs quantified the highest number of times among the 41 PAs not included in the Commission list. The focus was put on 'Lucerne, alfalfa' where a total of 131 samples were reported with at least one PA quantified (see **Table 6**); among them the 50 samples with the highest levels were selected. In these samples, heliosupine and, above all, acetylheliosupine contributed significantly to the total levels of PAs. Acetylheliosupine was quantified in 18 out of these 50 samples, in several occasions with

contribution above 40% (max = 57%); heliosupine was also quantified in 18 samples, in several with a contribution above 20% of the total PA levels (max = 27%).

The CONTAM Panel is of the opinion that a very limited number of feed samples are available to carry out a comprehensive evaluation of the PAs most typically present in feed. Furthermore, they come from one country and may be locally produced (grass, alfalfa). As a result, specific weeds present in these products may not be representative for those growing in other parts of the EU, like in the South or at higher altitudes. Based on this, it is difficult to conclude on which PAs should be monitored when analysing feed samples. Overall, a recommendation is given to analyse, at least, the 17 PAs proposed for food. Likewise, and as proposed for food, PAs other than those included in the proposed list should be also monitored to better understand the occurrence of PAs in feed.

2.4. Uncertainty analysis

Uncertainties associated to the estimates of dietary exposure to PAs have been already described (EFSA, 2016). In brief, the main uncertainties refer the large proportion of left-censored data, the fact that not all samples reported analytical data for all 28 PAs, and to the presence of an important number of both eating occasions and occurrence data on unspecified tea and herbs for infusions. Likewise, there is uncertainty on how analytical methods (extraction) represent the different ways consumers prepare tea and herbal infusions. In addition, the fact that many other PAs, not routinely monitored or not yet identified, could also be present in food may lead to an underestimation of the exposure levels. Overall, the dietary exposure to PAs calculated was likely to overestimate the exposure levels of the European population.

There are also uncertainties linked to the assessment of the PAs present in feed; the number of samples was very limited and collected in just one country so they may not be representative especially considering the role of weeds growing specifically in certain parts of Europe.

Regarding the hazard characterisation, the CONTAM Panel confirmed the uncertainties already identified in the 2011 opinion (EFSA CONTAM Panel, 2011), and noted additional uncertainties in particular related to the data sets used for the dose–response analysis for the characterisation of the carcinogenic hazard (see Section 2.1). However, the Panel confirmed that the main uncertainties remain considering the lack of toxicological data on most of the PAs of relevance for food and feed contamination. As already concluded in 2011, the CONTAM Panel confirmed that the carcinogenic potency of many PAs present in food is expected to be lower than the potency of the two PAs with available long term studies, lasiocarpine and riddelliine. Therefore, basing the cumulative risk assessment of PAs on an RP derived from riddelliine without correcting for individual potencies is considered as a conservative approach. In relation to the acute risk assessment, the CONTAM Panel noted substantial uncertainties in the available human data hindering the possibility to establish an ARfD.

3. Conclusions

- In view of the updated guidance of the EFSA Scientific Committee on the use of Benchmark dose in risk assessment, the CONTAM Panel updated the BMD analysis of the available long-term studies on lasiocarpine and riddelliine performed in its previous risk assessment. Using model averaging, the Panel calculated the BMD confidence interval and selected the $BMDL_{10}$ of 237 μg/kg bw per day for increase in the incidence of liver haemangiosarcoma in female rats exposed to riddelliine as the RP for chronic risk assessment.
- The CONTAM Panel updated the risk characterisation performed in its scientific opinion published in 2011 considering the updated RP and most recent exposure levels calculated in the EFSA report of 2016 considering data in honey, teas, herbal infusions and food supplements.
- In line with its previous opinion, considering the genotoxic and carcinogenic nature of PAs, the CONTAM Panel applied a MOE approach to the cumulative chronic exposure levels of PAs. The EFSA Scientific Committee concluded that, for substances that are both genotoxic and carcinogenic, an MOE of 10,000 or higher, based on a $BMDL_{10}$ from an animal study, and taking into account overall uncertainties in the interpretation, would be of low concern from a public health point of view.

- MOEs considering chronic mean exposure levels in all consumers ranged from > 10,000,000 to about 4,900 (min LB–max UB across dietary surveys and age classes) in the younger age classes and from > 1,000,000 to 5,700 in adults. When considering high (95th percentile) consumption, MOEs ranged from > 10,000,000 to about 1,100, and from > 200,000 to about 1,900 for the younger and adult age classes, respectively.
- In the case of the chronic mean exposure levels calculated in consumers only of different types of teas and herbal infusions, MOEs ranged from > 1,000,000 to 4,300 and from > 1,000,000 to 1,000 for the adult and young population, respectively. MOEs calculated at 95th percentile of consumption ranged from 395,000 to 1,500 and from 43,000 to 770 for the adult and young population, respectively. Lower MOEs were calculated for the consumption of camomile flowers in particular in the young population, and for rooibos leaves both for the adult and young population.
- In the case of the chronic mean exposure levels calculated in consumers only of retail honey, MOEs ranged between > 1,000,000 and 32,000 and between 593,000 and 13,500 for adults at mean and 95th percentile consumption, respectively. For the younger groups of the population, MOEs ranged between 790,000 and 8,800 and between 339,000 and 7,600 for mean and 95th percentile consumption, respectively.
- Overall, the CONTAM Panel concluded that the MOEs calculated for all consumers in the mean and high (95th percentile) consumption scenarios indicate a possible concern for human health. In particular, a concern was expressed for frequent and high consumers of teas or herbal infusions.
- The CONTAM Panel assessed also the acute/short-term risks, considering the dietary acute exposure levels estimated in the 2016 EFSA report and the lowest known dose range of 1–3 mg PA/kg bw per day, at which acute/short-term adverse effects have been reported in humans.
- Acute exposure considering the simultaneous presence of high contamination levels in all the different food commodities ranged from 1 to 300 ng/kg bw per day and from 6 to 170 ng/kg bw per day for mean consumers in the younger age classes (infants–adolescents) and adults, respectively. Exposure for the 95th percentile consumption levels was well below 1 μg/kg bw per day in all age classes. In view of the margin of more than three orders of magnitude between these exposure levels and the lowest known dose range associated with human acute/short-term adverse effects, the CONTAM Panel concluded that there is a low risk related to acute dietary exposure to PAs through the consumption of teas, herbal infusions and honey.
- Acute or short-term exposure to PAs related to the consumption of food supplements was estimated to vary considerably depending on the type of supplement. Consumption of PA producing plant extracts to be consumed following infusion led to exposure levels as high as 890 ng/kg bw per day. Ingestion of one tablet/capsule based on PA-producing plants corresponded to estimates of acute/short-term exposure levels of about 800 or 1,800 μg/kg bw per day. Acute/short-term exposure through the consumption of pollen-based supplements showed much lower exposure estimates in the range of 3–44 ng/kg bw per day.
- The CONTAM Panel concluded that the consumption of herbal food supplements based on PA-producing plants could reach acute/short-term exposure levels in the range of doses associated with severe acute/short-term effects in humans. In view of the uncertainty on the possible toxicity levels of PAs in humans and of the severity of the effects, the CONTAM Panel concluded that exposure levels less than 100 times lower than the aforementioned dose range of 1–3 mg PA/kg bw per day may be associated with the risk of acute/short-term effects.
- Consumption of pollen-based supplements is not considered to pose acute risks to human health.
- Based on the current data set, the CONTAM Panel proposed a set of 17 PAs to be monitored in food, namely: intermedine/lycopsamine, intermedine-*N*-oxide/lycopsamine-*N*-oxide, senecionine/senecivernine, senecionine-*N*-oxide/senecivernine-*N*-oxide, seneciphylline, seneciphylline-*N*-oxide, retrorsine, retrorsine-*N*-oxide, echimidine, echimidine-*N*-oxide, lasiocarpine, lasiocarpine-*N*-oxide, and senkirkine.
- The CONTAM Panel acknowledged that the number of feed samples was very limited to carry out a comprehensive evaluation of the PAs most typically present in feed. However, while expecting to have more representative data in the future, the Panel considered appropriate to monitor at least the 17 PAs proposed for food also in feed.

- The list of PAs proposed for monitoring in food and feed is not expected to cover all possible PAs that may be present in the different commodities, but to include the most relevant PAs considering both their contribution to the total levels and their possible toxicological potencies. This approach is expected to facilitate the monitoring of PAs without compromising a high level of consumer protection.

4. Recommendations

- There is a need for toxicological data relating to the PAs most commonly found in food. In particular information on the toxicokinetics, metabolic activation and carcinogenic potency of the individual PAs would allow substantial refinement of the risk assessment.
- Ongoing efforts should continue to collect analytical data on the occurrence of PAs in relevant food and feed commodities, as well as in herbal food supplements.
- Based on standard availability, PAs other than those included in the proposed list of 17 PAs should be also monitored to better understand the occurrence of PAs in food and feed.
- More sensitive and selective analytical methods should be developed to assess the presence of PAs in food and feed and to decrease the uncertainties in the exposure assessment.

References

BfR (Bundesinstitut für Risikobewertung), 2013. Pyrrolizidine alkaloids in herbal teas and teas. Opinion No. 018/2013. Available online: http://www.bfr.bund.de/cm/349/pyrrolizidine-alkaloids-in-herbal-teas-and-teas.pdf

Chen L, Mulder PPJ, Louisse J, Peijnenburg A, Wesseling S and Rietjens IMCM, 2017. Risk assessment for pyrrolizidine alkaloids detected in (herbal) teas and plant food supplements. Regulatory Toxicology and Pharmacology, 86, 292–302.

Crews C, 2013. Methods for analysis of pyrrolizidine alkaloids. In: Ramawat KG, Merillon JM (eds.). *Natural Products*. Springer-Verlag: Berlin & Heidelberg, Germany. pp. 1049–1068.

Dalefield RR, Gosse MA and Mueller U, 2016. A 28-day study of echimidine and lasiocarpine in Wistar rats. Regulatory Toxicology and Pharmacology, 81, 146–154.

EFSA (European Food Safety Authority), 2005. Opinion of the Scientific Committee on a request from EFSA related to A Harmonised Approach for Risk Assessment of substances which are both genotoxic and carcinogenic. EFSA Journal 2005;3(10):282, 33 pp. https://doi.org/10.2903/j.efsa.2005.282

EFSA (European Food Safety Authority), 2011. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal 2011;9(3):2097, 34 pp. https://doi.org/10.2903/j.efsa.2011.2097

EFSA (European Food Safety Authority), 2016. Dietary exposure assessment to pyrrolizidine alkaloids in the European population. EFSA Journal 2016;14(8):4572, 50 pp. https://doi.org/10.2903/j.efsa.2016.4572

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2011. Scientific Opinion on Pyrrolizidine alkaloids in food and feed. EFSA Journal 2011;9(11):2406, 134 pp. https://doi.org/10.2903/j.efsa.2011.2406

EFSA Scientific Committee, 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 32 pp. https://doi.org/10.2903/j.efsa.2012.2579

EFSA Scientific Committee, 2017. Update: use of the benchmark dose approach in risk assessment. EFSA Journal 2017;15(1):4658, 41 pp. https://doi.org/10.2903/j.efsa.2017.4658

El-Shazly A and Wink M, 2014. Diversity of pyrrolizidine alkaloids in the boraginaceae structures, distribution, and biological properties. Diversity, 6, 188–282.

FAO/WHO (Joint FAO/WHO Expert Committee on Food Additives), 2015. Summary and Conclusions of the Eightieth meeting, Rome 16–25 June 2015. Available online: http://www.fao.org/fileadmin/user_upload/agns/pdf/jecfa/Summary_report_of_the_80th_JECFA_meeting.pdf

IARC (International Agency for Research on Cancer), 1983. Some Food Additives, Feed Additives and Naturally Occurring Substances. IARC Monographs on Evaluation of Carcinogenic Risks to Humans 31, WHO, Lyon, France.

IARC (International Agency for Research on Cancer), 1987. IARC monographs on evaluation of carcinogenic risks to humans. 3 Volumes 1–42, Supplement 7, WHO, Lyon, France.

IARC (International Agency for Research on Cancer), 2002. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. IARC Monographs on Evaluation of Carcinogenic Risks to Humans, 82, WHO, Lyon, France. Available online: http://monographs.iarc.fr/ENG/Monographs/vol82/mono82.pdf

Merz K-H and Schrenk D, 2016. Interim relative potency factors for the toxicological risk assessment of pyrrolizidine alkaloids in food and herbal medicine. Toxicology Letters, 263, 44–57.

Mulder PPJ, López Sánchez P, These A, Preiss-Weigert A and Castellari M, 2015. Occurrence of Pyrrolizidine Alkaloids in food. EFSA Supporting Publication 2015; 12(8):EN-859, 114 pp. https://doi.org/10.2903/sp.efsa.2015.en-859

Nedelcheva A, Kostova N and Sidjimov A, 2015. Pyrrolizidine alkaloids in Tussilago farfara from Bulgaria. Biotechnology and Biotechnological Equipment, 29 (sup 1), S1–S7.
NTP (National Toxicology Program), 1978. Bioassay of lasiocarpine for possible carcinogenicity. NTP Technical Report, 39, 1–66. Available online: https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr039.pdf
NTP (National Toxicology Program), 2003. Toxicology and carcinogenesis studies of riddelliine. NTP Technical Report 508. Available online: https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr508.pdf
SCOFCAH (Standing Committee on the Food Chain and Animal Health), 2014. Compilation of agreed monitoringrecommendations as regards the presence of mycotoxins and plant toxins in food. Available online: http://ec.europa.eu/food/safety/docs/cs_contaminants_catalogue_plant_toxins_compilation_agreed_monitoring_en.pdf
Wheeler MW and Bailer AJ, 2008. Model averaging software for dichotomous dose response risk estimation. Journal of Statistical Software, 26, 15. https://doi.org/10.18637/jss.v026.i05
Zwicker GM, Eyster RC, Sells DM and Gass JH, 1995. Spontaneous vascular neoplasms in aged Sprague-Dawley rats. Toxicologic Pathology, 23, 518–526.

Abbreviations

AIC	Akaike information criterion
ARfD	acute reference dose
bw	body weight
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDU	benchmark dose upper confidence limit
BMR	benchmark response
CI	confidence interval
CONTAM	EFSA Panel on Contaminants in the Food Chain
HPLC–MS/MS	high-performance liquid chromatography – tandem mass spectrometry
HVOD	hepatic veno-occlusive disease
IARC	International Agency for Research on Cancer
i.p.	intraperitoneal
i.v.	Intravenous
JECFA	Joint FAO/WHO Expert Committee on Food Additives and Contaminants
LB	lower bound
LC–MS/MS	liquid chromatography with tandem mass spectrometry
LD_{50}	lethal dose, median
LOD	limit of detection
LOQ	limit of quantification
MOE	Margin of Exposure
NTP	National Toxicology Programme
PA	pyrrolizidine alkaloids
PANO	pyrrolizidine alkaloid-*N*-oxide
RP	reference point
RPF	relative potency factor
s.c.	subcutaneous
THIE	Tea & Herbal Infusions Europe
UB	upper bound

Appendix A – Benchmark dose modelling of incidence of liver haemangiosarcoma in male rats exposed to lasiocarpine (NTP, 1978)

A. Data description

As already outlined in the previous EFSA opinion (EFSA CONTAM Panel, 2011), reported lasiocarpine levels administered in the diet were converted to doses by considering average body weight and daily food intake of 400 g and 20 g, respectively. This corresponds to the default conversion factor of 0.05 recommended by the EFSA Scientific Committee guidance (2012) for chronic rat studies.

Dose (μg/kg bw per day)	Incidence liver haemangiosarcoma	N
0	0	23
350	5	24
750	11	23
1,500	13	23

N: number of animals; bw: body weight.

B. Selection of benchmark response

A default benchmark response (BMR) of 10% (extra risk compared with the background risk) and a two-sided 90% confidence interval of the BMD were selected as recommended by EFSA Scientific Committee (2017). Additional calculations were performed applying a BMR of 30% for comparing carcinogenic potencies of lasiocarpine and riddelliine.

C. Software used and specifications

- Fitting benchmark dose models was based on the R-package proast61.3.
- Averaging results from multiple fitted benchmark dose models was based on the methodology in Wheeler and Bailer (2008).
- The default set of fitted models was applied as recommended by EFSA Scientific Committee (2017)
- Selection of the BMD confidence interval and the BMDL was carried out following the flow chart of EFSA Scientific Committee (2017)

D. Results

Model	Number of parameters	Log-likelihood	AIC	BMD_{10}[(a)]	$BMDL_{10}$[(a)]	$BMDU_{10}$[(a)]	Converged	Accepted AIC
Null	1	−57.71	117.42	NA	NA	NA	Yes	
Full	4	−43.95	95.90	NA	NA	NA	Yes	
Logistic	2	−48.17	100.34	392.86	301.85	510.82	Yes	No
Probit	2	−47.89	99.78	388.11	281.08	489.20	Yes	No
Log-logistic	3	−44.25	94.50	134.32	4.81	297.70	Yes	Yes
Log-probit	3	−44.23	94.46	151.06	7.36	309.78	Yes	Yes
Weibull	3	−44.34	94.68	103.34	1.70	271.73	Yes	Yes
Gamma	3	−44.36	94.72	99.41	0.65	283.22	Yes	Yes
Two-stage	3	−44.50	95.00	157.76	117.10	218.95	Yes	Yes

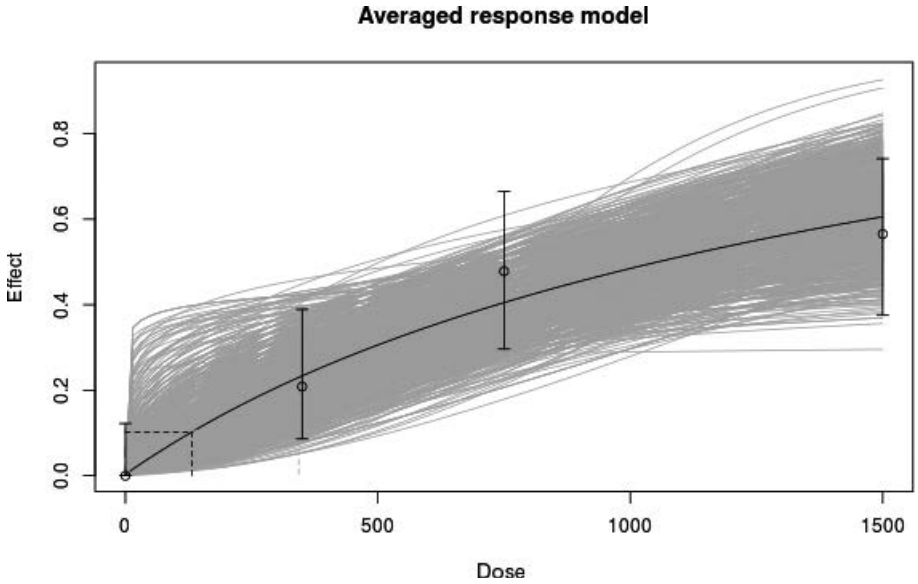
AIC: Akaike information criterion; BMD: benchmark dose; BMDL: benchmark dose lower confidence limit; BMDU: benchmark dose upper confidence limit.
(a): Results expressed as μg/kg bw per day.

	Logistic	Probit	Log-logistic	Log-probit	Weibull	Gamma	Two-stage
Estimated model weights	0.01	0.02	0.21	0.22	0.19	0.19	0.16

Using the parametric bootstrap with a total of 1,000 generated data sets, the BMDL and the BMDU were the 5th and 95th percentile of all parametric bootstrap BMD values, respectively.

Estimates in μg/kg bw per day based on the model averaging (see EFSA Scientific Committee, 2017):

BMD_{10}	$BMDL_{10}$	$BMDU_{10}$
131.38	8.34	343.32



When applying a BMR of 30%, the following results were obtained

Model	Number of parameters	Log-likelihood	AIC	BMD_{30} [(a)]	$BMDL_{30}$ [(a)]	$BMDU_{30}$ [(a)]	Converged	Accepted AIC
Null	1	−57.71	117.42	NA	NA	NA	Yes	
Full	4	−43.95	95.90	NA	NA	NA	Yes	
Logistic	2	−48.17	100.34	880.86	711.16	1,166.91	Yes	No
Probit	2	−47.86	99.72	857.49	698.66	1,153.10	Yes	No
Log-logistic	3	−44.25	94.50	470.78	158.26	705.30	Yes	Yes
Log-probit	3	−44.23	94.46	470.49	162.65	695.34	Yes	Yes
Weibull	3	−44.34	94.68	469.42	133.92	726.08	Yes	Yes
Gamma	3	−44.36	94.72	473.39	119.38	727.37	Yes	Yes
Two-stage	3	−44.50	95.00	534.05	396.43	741.21	Yes	Yes

AIC: Akaike information criterion; BMD: benchmark dose; BMDL: benchmark dose lower confidence limit; BMDU: benchmark dose upper confidence limit.
(a): Results expressed as μg/kg bw per day.

	Logistic	Probit	Log-logistic	Log-probit	Weibull	Gamma	Two-stage
Estimated model weights	0.01	0.02	0.21	0.22	0.19	0.19	0.16

Using the parametric bootstrap with a total of 1,000 generated data sets, the BMDL and the BMDU were the 5th and 95th percentile of all parametric bootstrap BMD values, respectively.

Estimates in μg/kg bw per day based on the model averaging (see EFSA Scientific Committee, 2017):

BMD_{30}	$BMDL_{30}$	$BMDU_{30}$
490.88	210.5	810.85

Appendix B – Benchmark dose modelling of incidence of liver haemangiosarcoma in female rats exposed to riddelliine (NTP, 2003)

A. Data description

As already discussed in the previous EFSA opinion (EFSA CONTAM Panel, 2011), reported riddelliine doses were corrected by a factor of 5/7 to account for the exposure regime applied in the study (5 days of exposure per week) were converted to doses by considering average bw and daily food intake of 400 g and 20 g, respectively. This corresponds to the default conversion factor of 0.05 recommended by the EFSA Scientific Committee guidance (2012) for chronic rat studies.

Dose (μg/kg bw per day)	Incidence liver haemangiosarcoma	N
0	0	50
7	0	50
24	0	50
71	0	50
236	3	50
714	38	50

N: number of animals.

B. Selection of benchmark response

A default benchmark response (BMR) of 10% (extra risk compared with the background risk) and a 90% interval around the BMD were selected as recommended by EFSA Scientific Committee (2017). Additional calculations were performed applying a BMR of 30% for comparing carcinogenic potencies of lasiocarpine and riddelliine.

C. Software used and specifications

- Fitting benchmark dose models was based on the R-package proast61.3.
- Averaging results from multiple fitted benchmark dose models was based on the methodology in Wheeler and Bailer (2008).
- Default set of fitted models were applied as recommended by EFSA Scientific Committee (2017)
- Selection of BMDL was carried out following the flow chart of EFSA Scientific Committee (2017)

D. Results

Model	Number of parameters	Log-likelihood	AIC	BMD_{10}[(a)]	$BMDL_{10}$[(a)]	$BMDU_{10}$[(a)]	Converged	Accepted AIC
Null	1	−119.66	241.32	NA	NA	NA	Yes	
Full	6	−38.90	89.80	NA	NA	NA	Yes	
Logistic	2	−40.32	84.64	362.77	298.90	430.74	Yes	Yes
Probit	2	−39.63	83.26	327.91	270.55	385.59	Yes	Yes
Log-logistic	3	−38.95	83.90	278.32	216.29	345.24	Yes	Yes
Log-probit	3	−38.90	83.80	269.90	215.09	323.21	Yes	Yes
Weibull	3	−39.00	84.00	290.30	218.19	366.26	Yes	Yes
Gamma	3	−38.92	83.84	277.13	215.62	336.89	Yes	Yes
Two-stage	3	−41.12	88.24	207.97	182.26	239.53	No	No

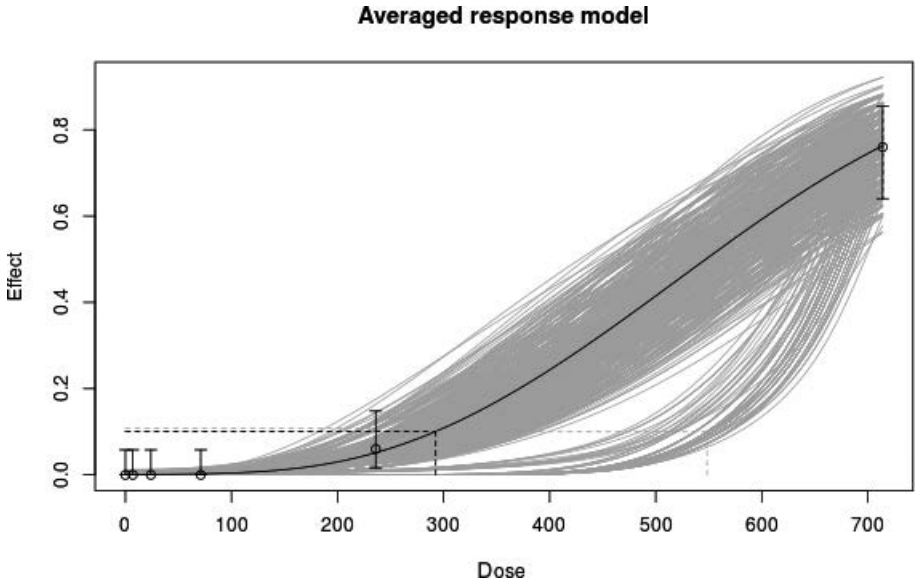
AIC: Akaike information criterion; BMD: benchmark dose; BMDL: benchmark dose lower confidence limit; BMDU: benchmark dose upper confidence limit.
(a): results expressed as μg/kg bw per day.

	Logistic	Probit	Log-logistic	Log-probit	Weibull	Gamma
Estimated model weights	0.11	0.23	0.16	0.17	0.16	0.17

Using the parametric bootstrap with a total of 1,000 generated data sets, the BMDL and the BMDU were the 5th and 95th percentile of all parametric bootstrap BMD values, respectively.

Estimates in μg/kg bw per day based on the model averaging (see EFSA Scientific Committee, 2017):

BMD_{10}	$BMDL_{10}$	$BMDU_{10}$
292.53	236.58	548.31



When applying a BMR of 30%, the following results were obtained

Model	Number of parameters	Log-likelihood	AIC	BMD_{30} [a]	$BMDL_{30}$ [a]	$BMDU_{30}$ [a]	Converged	Accepted AIC
Null	1	−119.66	241.32	NA	NA	NA	Yes	
Full	6	−38.90	89.80	NA	NA	NA	Yes	
Logistic	2	−40.32	84.64	501.48	447.86	553.76	Yes	Yes
Probit	2	−39.63	83.26	473.57	423.56	525.77	Yes	Yes
Log-logistic	3	−38.95	83.90	406.60	344.26	472.04	Yes	Yes
Log-probit	3	−38.90	83.80	390.94	335.64	447.35	Yes	Yes
Weibull	3	−39.00	84.00	442.09	375.50	506.25	Yes	Yes
Gamma	3	−38.92	83.84	411.12	353.44	468.78	Yes	Yes
Two-stage	3	−41.12	88.24	382.65	335.35	440.72	No	No

AIC: Akaike information criterion; BMD: benchmark dose; BMDL: benchmark dose lower confidence limit; BMDU: benchmark dose upper confidence limit.
(a): results expressed as μg/kg bw per day.

	Logistic	Probit	Log-logistic	Log-probit	Weibull	Gamma
Estimated model weights	0.11	0.23	0.16	0.17	0.16	0.17

Using the parametric bootstrap with a total of 1,000 generated data sets, the BMDL and the BMDU were the 5th and 95th percentile of all parametric bootstrap BMD values, respectively.

Estimates in μg/kg bw per day based on the model averaging (see EFSA Scientific Committee, 2017):

BMD_{30}	$BMDL_{30}$	$BMDU_{30}$
434.91	373.01	622.37

Appendix C – Margin of Exposure tables

Table C.1: All consumers exposure levels calculated in the EFSA report on dietary exposure assessment to pyrrolizidine alkaloids (PAs) (EFSA, 2016), using occurrence data set from THIE (Scenario B, see Section 1.3.2), and related Margin of Exposure (MOEs) using the Reference Point of 237 μg/kg bw per day for the sum of all 1,2-unsaturated PAs

Age class		Mean dietary exposure (ng/kg bw per day)						MOEs Mean dietary exposure					
		Lower bound[a]			Upper bound[a]			Lower bound			Upper bound		
	N	Min	Median	Max	Min	Median	Max	Min	Median	Max	Min	Median	Max
Infants	6	0.00	0.60	5.50	0.00	3.60	26.60	(> 1,000,000)	395,000	43,091	(> 1,000,000)	65,833	8,910
Toddlers	10	0.00	1.00	6.10	0.00	4.60	29.80	(> 1,000,000)	237,000	38,852	(> 1,000,000)	51,522	7,953
Other children	18	0.20	1.20	4.40	1.00	5.20	23.70	1,185,000	197,500	53,864	237,000	45,577	10,000
Adolescents	17	0.20	0.70	3.40	0.50	4.40	18.10	1,185,000	338,571	69,706	474,000	53,864	13,094
Adults	17	0.10	1.20	3.70	0.40	8.10	22.60	2,370,000	197,500	64,054	592,500	29,259	10,487
Elderly	14	0.70	1.80	5.40	3.40	9.80	31.60	338,571	131,667	43,889	69,706	24,184	7,500
Very elderly	12	0.90	1.80	5.70	4.30	10.90	33.40	263,333	131,667	41,579	55,116	21,743	7,096

Age class		95th percentile dietary exposure[b] (ng/kg bw per day)						MOEs 95th percentile dietary exposure					
		Lower bound[a]			Upper bound[a]			Lower bound			Upper bound		
	N	Min	Median	Max	Min	Median	Max	Min	Median	Max	Min	Median	Max
Infants	5	0.00	_[c]	19.00	0.00	_[c]	106.20	(> 1,000,000)		12,474	(> 1,000,000)		2,232
Toddlers	7	0.00	7.60	23.30	0.00	45.60	131.30	(> 1,000,000)	31,184	10,172	(> 1,000,000)	5,197	1,805
Other children	18	1.30	7.00	14.30	6.30	26.70	77.00	182,308	33,857	16,573	37,619	8,876	3,078
Adolescents	17	0.80	3.70	13.10	2.40	18.50	64.90	296,250	64,054	18,092	98,750	12,811	3,652
Adults	17	0.90	5.40	14.70	1.90	33.70	78.10	263,333	43,889	16,122	124,737	7,033	3,035
Elderly	14	3.00	6.70	14.70	15.90	37.20	78.80	79,000	35,373	16,122	14,906	6,371	3,008
Very elderly	9	4.00	8.20	15.90	18.20	33.90	76.90	59,250	28,902	14,906	13,022	6,991	3,082

bw: body weight.
(a): Estimates were rounded to one decimal figure.
(b): The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may be not statistically robust (EFSA, 2011). Those estimates were not included in the table.
(c): A minimum number of six dietary surveys is required to estimate a statistically robust median (EFSA, 2011).

Table C.2: Consumers only exposure levels calculated in the EFSA report on dietary exposure assessment to pyrrolizidine alkaloids (PAs) (EFSA, 2016), using occurrence data set from Article 36 project and EU Member States (Scenario A, see Section 1.3.2), and related Margin of Exposure (MOEs) using the Reference Point of 237 μg/kg bw per day for the sum of all 1,2-unsaturated PAs

	Adult consumers															
	Mean exposure				P95 exposure				MOEs (Mean exposure)				MOEs (P95 exposure)			
	Min LB	Max LB	Min UB	Max UB	Min LB	Max LB	Min UB	Max UB	Min LB	Max LB	Min UB	Max UB	Min LB	Max LB	Min UB	Max UB
Tea and herbs for infusions, unspecified	0.2	39.6	0.2	54.7	0.6	114.4	0.8	158.1	1,185,000	5,985	1,185,000	4,333	395,000	2,072	296,250	1,499
Tea, unspecified	0.9	22.2	1.5	37	6	53.7	10	89.5	263,333	10,676	158,000	6,405	39,500	4,413	23,700	2,648
Tea unspecified, decaffeinated	0.5	2.5	2.3	12.6	6.3	6.3	31.5	31.5	474,000	94,800	103,043	18,810	37,619	37,619	7,524	7,524
Black tea, infusion	1.9	32.2	2.5	42.6	15.9	70.3	21.1	93.1	124,737	7,360	94,800	5,563	14,906	3,371	11,232	2,546
Green tea, infusion	2.4	15.4	4.8	30.7	15.4	41.8	30.7	83.5	98,750	15,390	49,375	7,720	15,390	5,670	7,720	2,838
Camomile flowers	1.9	14.1	2.7	19.6	39.9	39.9	55.7	55.7	124,737	16,809	87,778	12,092	5,940	5,940	4,255	4,255
Peppermint	0.7	34	0.8	42					338,571	6,971	296,250	5,643				
Rooibos leaves	11	36	12.6	41.3	32.9	96.4	37.8	110.6	21,545	6,583	18,810	5,738	7,204	2,459	6,270	2,143
Tea for infants and young children	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Honey	0.1	3.9	0.3	7.4	0.4	9.3	0.8	17.6	2,370,000	60,769	790,000	32,027	592,500	25,484	296,250	13,466
	Young consumers															
	Mean exposure				P95 exposure				MOEs (Mean exposure)				MOEs (P95 exposure)			
	Min LB	Max LB	Min UB	Max UB	Min LB	Max LB	Min UB	Max UB	Min LB	Max LB	Min UB	Max UB	Min LB	Max LB	Min UB	Max UB
Tea and herbs for infusions, unspecified	0.6	165	0.8	228	5.5	222.2	7.6	307	395,000	1,436	296,250	1,039	43,091	1,067	31,184	772
Tea, unspecified	0.6	33.9	1	56.5	14.7	93	24.5	155	395,000	6,991	237,000	4,195	16,122	2,548	9,673	1,529
Tea unspecified, decaffeinated	0.4	2	2.1	9.9					592,500	118,500	112,857	23,939				
Black tea, infusion	1.5	41.4	2	54.9	44.4	64.4	58.8	85.3	158,000	5,725	118,500	4,317	5,338	3,680	4,031	2,778
Green tea, infusion	1.2	11.5	2.4	23					197,500	20,609	98,750	10,304				
Camomile flowers	8.4	32.3	11.7	45.1					28,214	7,337	20,256	5,255				
Peppermint	0.7	29.9	0.8	37	61.9			74.6	338,571	7,926	296,250	6,405	3,829			3,177

	Young consumers															
	Mean exposure				P95 exposure				MOEs (Mean exposure)				MOEs (P95 exposure)			
	Min LB	Max LB	Min UB	Max UB	Min LB	Max LB	Min UB	Max UB	Min LB	Max LB	Min UB	Max UB	Min LB	Max LB	Min UB	Max UB
Rooibos leaves	13.4	70.2	15.4	80.5					17,687	3,376	15,390	2,944				
Tea for infants and young children	0.2	10.6	0.4	24.8					1,185,000	22,358	592,500	9,556				
Honey	0.3	14.2	0.6	27	0.7	16.4	1.4	31.1	790,000	16,690	395,000	8,778	338,571	14,451	169,286	7,621

LB: lower bound; UB: upper bound.

Appendix D – Hypothetical chronic exposure estimates to PAs across different dietary surveys considering consumers only

	Concentration of PAs[d] (μg/kg per μg/L)	Young population[a]		Adult population[b]	
		Mean exposure	95th exposure[c]	Mean exposure	95th exposure[c]
		ng/kg bw per day		ng/kg bw per day	
Tea and herbs for infusions, unspecified	19/0.25	0.03–7.5	0.25–10.1	0.01–1.8	0.03–5.2
Tea unspecified	19/0.25	0.05–2.8	1.2–7.8	0.08–1.9	0.5–4.5
Tea unspecified, decaffeinated	19/0.25	0.35–1.7	–	0.38–2.1	5.3
Black tea, infusion	19/0.25	0.10–2.8	3.0–4.4	0.13–2.2	1.08–4.8
Green tea, infusion	19/0.25	0.13–1.2	–	0.25–1.6	1.60–4.4
Camomile flowers	19/0.25	0.55–2.1	–	0.13–0.9	2.6
Peppermint	19/0.25	0.03–1.1	2.3	0.03–1.3	–
Rooibos leaves	19/0.25	0.55–2.9	–	0.45–1.5	1.35–4.0
Tea for infants and young children	19/0.25	0.08–4.4	–	–	–

PA: pyrrolizidine alkaloid; bw: body weight.
(a): Young population comprises the age classes 'Infants', 'Toddlers' and 'Other children' across the different dietary surveys.
(b): Adult population comprises the age classes 'Adults', 'Elderly' and 'Very elderly' across the different dietary surveys.
(c): The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011). Those estimates were not included in this table.
(d): Hypothetical concentration of PAs assuming that the 17 selected PAs were all left-censored data and the analytical method used reported the lowest LOQs as provided in Table 12 of the 2016 EFSA scientific report on dietary exposure to PAs. Levels in μg/L for tea/herbal infusions are obtained using 2 g of dry product in 150 mL of water.

Safety Data Sheet
acc. to OSHA HCS

Printing date 04/20/2018 Reviewed on 04/20/2018

*
1 Identification

· **Product identifier**

· **Trade name: Peppermint (Mentha piperita)**

· **Article number:**
3614, 3613, 5628, 24223, 345905, 200701, 200712, 200715, 200722, 361302, 361303, 361305, 361308, 361310, 361311, 361313, 361317, 361329, 361376, 3613515, 3613531, 361402, 361403, 361405, 361408, 361410, 361411, 361413, 361415, 361417, 361422, 361429, 361476, 3614515, 3614531, 562805, 562808, 562810, 562829, 110780

· **CAS Number:**
8006-90-4

· **Details of the supplier of the safety data sheet**

· **Manufacturer/Supplier:**
Young Living
3125 Executive Parkway
Lehi, UT 84043
productsafety@youngliving.com

· **Information department:** Health Sciences and Product Safety

· **Emergency telephone number:**
Chemtrec (US): (800) 424-9300
Chemtrec (Outside US): (703) 527-3887 (Collect calls accepted.)

2 Hazard(s) identification

· **Classification of the substance or mixture**

GHS08 Health hazard

Asp. Tox. 1 H304 May be fatal if swallowed and enters airways.

GHS07

Skin Irrit. 2 H315 Causes skin irritation.
Eye Irrit. 2A H319 Causes serious eye irritation.
Skin Sens. 1 H317 May cause an allergic skin reaction.

Flam. Liq. 4 H227 Combustible liquid.

· **Label elements**

· **GHS label elements** The substance is classified and labeled according to the Globally Harmonized System (GHS).

· **Hazard pictograms**

GHS07 GHS08

(Contd. on page 2)

Safety Data Sheet
acc. to OSHA HCS

Printing date 04/20/2018 Reviewed on 04/20/2018

Trade name: Peppermint (Mentha piperita)

(Contd. of page 1)

· **Signal word** Danger

· **Hazard-determining components of labeling:**
Peppermint oil
· **Hazard statements**
Combustible liquid.
Causes skin irritation.
Causes serious eye irritation.
May cause an allergic skin reaction.
May be fatal if swallowed and enters airways.
· **Precautionary statements**
Keep away from flames and hot surfaces. – No smoking.
Avoid breathing dust/fume/gas/mist/vapors/spray
Wash thoroughly after handling.
Contaminated work clothing must not be allowed out of the workplace.
Wear protective gloves/protective clothing/eye protection/face protection.
If swallowed: Immediately call a poison center/doctor.
Specific treatment (see on this label).
Do NOT induce vomiting.
If on skin: Wash with plenty of water.
If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Take off contaminated clothing and wash it before reuse.
If skin irritation or rash occurs: Get medical advice/attention.
If eye irritation persists: Get medical advice/attention.
Wash contaminated clothing before reuse.
In case of fire: Use for extinction: CO2, powder or water spray.
Store in a well-ventilated place. Keep cool.
Store locked up.
Dispose of contents/container in accordance with local/regional/national/international regulations.
· **Classification system:**
· **NFPA ratings (scale 0 - 4)**

Health = 2
Fire = 2
Reactivity = 0

· **HMIS-ratings (scale 0 - 4)**

Health = 2
Fire = 2
Reactivity = 0

· **Other hazards**
· **Results of PBT and vPvB assessment**
· **PBT:** Not applicable.
· **vPvB:** Not applicable.

(Contd. on page 3)

Safety Data Sheet
acc. to OSHA HCS

Printing date 04/20/2018 Reviewed on 04/20/2018

Trade name: Peppermint (Mentha piperita)

(Contd. of page 2)

3 Composition/information on ingredients

· **Chemical characterization: Substances**
· **CAS No. Description**
8006-90-4 Peppermint oil

4 First-aid measures

· **Description of first aid measures**
· **General information:** Immediately remove any clothing soiled by the product.
· **After inhalation:**
Supply fresh air and to be sure call for a doctor.
In case of unconsciousness place patient stably in side position for transportation.
· **After skin contact:**
Immediately wash with water and soap and rinse thoroughly.
Dilute and rinse the skin with vegetable oil to dilute the essential oil
· **After eye contact:**
Rinse opened eye for several minutes under running water. If symptoms persist, consult a doctor.
Apply vegetable oil with a sterile cloth around the eye to dilute any excess essential oil.
· **After swallowing:** If symptoms persist consult doctor.
· **Information for doctor:**
· **Most important symptoms and effects, both acute and delayed** No further relevant information available.
· **Indication of any immediate medical attention and special treatment needed**
No further relevant information available.

5 Fire-fighting measures

· **Extinguishing media**
· **Suitable extinguishing agents:**
CO2, extinguishing powder or water spray. Fight larger fires with water spray or alcohol resistant foam.
· **Special hazards arising from the substance or mixture** No further relevant information available.
· **Advice for firefighters**
· **Protective equipment:** No special measures required.

6 Accidental release measures

· **Personal precautions, protective equipment and emergency procedures**
Wear protective equipment. Keep unprotected persons away.
· **Environmental precautions:** Do not allow to enter sewers/ surface or ground water.
· **Methods and material for containment and cleaning up:**
Absorb with liquid-binding material (sand, diatomite, acid binders, universal binders, sawdust).
Dispose contaminated material as waste according to item 13.
Ensure adequate ventilation.
· **Reference to other sections**
See Section 7 for information on safe handling.
See Section 8 for information on personal protection equipment.
See Section 13 for disposal information.
· **Protective Action Criteria for Chemicals**

· **PAC-1:**
Substance is not listed.

(Contd. on page 4)

Safety Data Sheet
acc. to OSHA HCS

Printing date 04/20/2018 Reviewed on 04/20/2018

Trade name: Peppermint (Mentha piperita)

(Contd. of page 3)

· **PAC-2:**
Substance is not listed.

· **PAC-3:**
Substance is not listed.

7 Handling and storage

· **Handling:**
· **Precautions for safe handling**
Ensure good ventilation/exhaustion at the workplace.
Prevent formation of aerosols.
· **Information about protection against explosions and fires:** Keep ignition sources away - Do not smoke.

· **Conditions for safe storage, including any incompatibilities**
· **Storage:**
· **Requirements to be met by storerooms and receptacles:** Store in a cool location.
· **Information about storage in one common storage facility:** Store in the dark away from heat.
· **Further information about storage conditions:** Keep receptacle tightly sealed.
· **Specific end use(s)** No further relevant information available.

8 Exposure controls/personal protection

· **Additional information about design of technical systems:** No further data; see item 7.

· **Control parameters**
· **Components with limit values that require monitoring at the workplace:** Not required.
· **Additional information:** The lists that were valid during the creation were used as basis.

· **Exposure controls**
· **Personal protective equipment:**
· **General protective and hygienic measures:**
Keep away from foodstuffs, beverages and feed.
Immediately remove all soiled and contaminated clothing.
Wash hands before breaks and at the end of work.
Avoid contact with the eyes and skin.
· **Breathing equipment:**
In case of brief exposure or low pollution use respiratory filter device. In case of intensive or longer exposure use respiratory protective device that is independent of circulating air.
· **Protection of hands:**

Protective gloves

The glove material has to be impermeable and resistant to the product/ the substance/ the preparation.
Due to missing tests no recommendation to the glove material can be given for the product/ the preparation/ the chemical mixture.
Selection of the glove material on consideration of the penetration times, rates of diffusion and the degradation
· **Material of gloves**
The selection of the suitable gloves does not only depend on the material, but also on further marks of quality and varies from manufacturer to manufacturer.

(Contd. on page 5)

Safety Data Sheet
acc. to OSHA HCS

Trade name: Peppermint (Mentha piperita)

(Contd. of page 4)

· **Penetration time of glove material**
The exact break through time has to be found out by the manufacturer of the protective gloves and has to be observed.
· **Eye protection:**

Tightly sealed goggles

9 Physical and chemical properties

· **Information on basic physical and chemical properties**	
· **General Information**	
· **Appearance:**	
Form:	Oily Liquid
Color:	Colorless
· **Odor:**	Characteristic
· **Odor threshold:**	Not determined.
· **pH-value:**	5.6
· **Change in condition**	
Melting point/Melting range:	Undetermined.
Boiling point/Boiling range:	Undetermined.
· **Flash point:**	74.5 °C (166.1 °F) Does not sustain combustion.
· **Flammability (solid, gaseous):**	Not applicable.
· **Decomposition temperature:**	Not determined.
· **Auto igniting:**	Not determined.
· **Danger of explosion:**	Not determined.
· **Explosion limits:**	
Lower:	Not determined.
Upper:	Not determined.
· **Vapor pressure:**	Not determined.
· **Density:**	0.899 g/mL
· **Relative density**	Not determined.
· **Vapor density**	Not determined.
· **Evaporation rate**	Not determined.
· **Solubility in / Miscibility with**	
Water:	Not miscible or difficult to mix.
· **Partition coefficient (n-octanol/water):**	Not determined.
· **Viscosity:**	
Dynamic:	Not determined.
Kinematic:	Not determined.
VOC content:	0.00 % 0.0 g/l / 0.00 lb/gl
Solids content:	0.0 %

(Contd. on page 6)

Safety Data Sheet
acc. to OSHA HCS

Printing date 04/20/2018 Reviewed on 04/20/2018

Trade name: Peppermint (Mentha piperita)

(Contd. of page 5)

· **Other information** No further relevant information available.

10 Stability and reactivity

· **Reactivity** No further relevant information available.
· **Chemical stability**
· **Thermal decomposition / conditions to be avoided:** No decomposition if used according to specifications.
· **Possibility of hazardous reactions** No dangerous reactions known.
· **Conditions to avoid** No further relevant information available.
· **Incompatible materials:** No further relevant information available.
· **Hazardous decomposition products:** No dangerous decomposition products known.

11 Toxicological information

· **Information on toxicological effects**
· **Acute toxicity:**

· **LD/LC50 values that are relevant for classification:**		
Oral	LD50	4.44 g/kg (rat)

· **Primary irritant effect:**
· **on the skin:** Irritant to skin and mucous membranes.
· **on the eye:** Irritating effect.
· **Sensitization:** Sensitization possible through skin contact.
· **Additional toxicological information:**
Peppermint: Choleretic, neurotoxicity, mucous membrane irritation (low risk). Contraindications (all routes): Cardiac fibrillation, G6PD deficiency, Do not apply to or near the face of infants and children. Contraindications (oral): Cholestasis. Cautions (oral): gastroesophageal reflux disease. Maximum adult daily oral dose 152 mg. Maximum dermal use level 5.4%. Menthol blocks cardiovascular calcium channels which could lead to a depressant effect on the heart. Menthol has caused neonatal jaundice in babies with a deficiency of the enzyme glucose-6-phosphate dehydrogenase. 200 mg/kg can produce signs of liver toxicity in rats. Oral LD50 in rats 4.44 g/kg.

· **Carcinogenic categories**

· **IARC (International Agency for Research on Cancer)**
Substance is not listed.

· **NTP (National Toxicology Program)**
Substance is not listed.

· **OSHA-Ca (Occupational Safety & Health Administration)**
Substance is not listed.

12 Ecological information

· **Toxicity**
· **Aquatic toxicity:** No further relevant information available.
· **Persistence and degradability** No further relevant information available.
· **Behavior in environmental systems:**
· **Bioaccumulative potential** No further relevant information available.
· **Mobility in soil** No further relevant information available.

(Contd. on page 7)

Safety Data Sheet
acc. to OSHA HCS

Printing date 04/20/2018 Reviewed on 04/20/2018

Trade name: Peppermint (Mentha piperita)

(Contd. of page 6)

· **Additional ecological information:**
· **General notes:**
Water hazard class 2 (Self-assessment): hazardous for water
Do not allow product to reach ground water, water course or sewage system.
Danger to drinking water if even small quantities leak into the ground.
· **Results of PBT and vPvB assessment**
· **PBT:** Not applicable.
· **vPvB:** Not applicable.
· **Other adverse effects** No further relevant information available.

13 Disposal considerations

· **Waste treatment methods**
· **Recommendation:**
Must not be disposed of together with household garbage. Do not allow product to reach sewage system.

· **Uncleaned packagings:**
· **Recommendation:** Disposal must be made according to official regulations.

*
14 Transport information

· **UN-Number**	
· **DOT, ADN, IMDG, IATA**	not regulated
· **UN proper shipping name**	
· **DOT, ADN, IMDG, IATA**	not regulated
· **Transport hazard class(es)**	
· **DOT, ADN, IMDG, IATA**	
· **Class**	not regulated
· **Packing group**	
· **DOT, IMDG, IATA**	not regulated
· **Environmental hazards:**	Not applicable.
· **Special precautions for user**	Not applicable.
· **Transport in bulk according to Annex II of MARPOL73/78 and the IBC Code**	Not applicable.
· **UN "Model Regulation":**	not regulated

15 Regulatory information

· **Safety, health and environmental regulations/legislation specific for the substance or mixture**
· **Sara**

· **Section 355 (extremely hazardous substances):**
Substance is not listed.

· **Section 313 (Specific toxic chemical listings):**
Substance is not listed.

(Contd. on page 8)

Safety Data Sheet
acc. to OSHA HCS

Trade name: Peppermint (Mentha piperita)

(Contd. of page 7)

· **TSCA (Toxic Substances Control Act):**
Substance is listed.

· **Proposition 65**

· **Chemicals known to cause cancer:**
Substance is not listed.

· **Chemicals known to cause reproductive toxicity for females:**
Substance is not listed.

· **Chemicals known to cause reproductive toxicity for males:**
Substance is not listed.

· **Chemicals known to cause developmental toxicity:**
Substance is not listed.

· **Carcinogenic categories**

· **EPA (Environmental Protection Agency)**
Substance is not listed.

· **TLV (Threshold Limit Value established by ACGIH)**
Substance is not listed.

· **NIOSH-Ca (National Institute for Occupational Safety and Health)**
Substance is not listed.

· **GHS label elements** The substance is classified and labeled according to the Globally Harmonized System (GHS).
· **Hazard pictograms**

GHS07 GHS08

· **Signal word** Danger

· **Hazard-determining components of labeling:**
Peppermint oil
· **Hazard statements**
Combustible liquid.
Causes skin irritation.
Causes serious eye irritation.
May cause an allergic skin reaction.
May be fatal if swallowed and enters airways.
· **Precautionary statements**
Keep away from flames and hot surfaces. – No smoking.
Avoid breathing dust/fume/gas/mist/vapors/spray
Wash thoroughly after handling.
Contaminated work clothing must not be allowed out of the workplace.
Wear protective gloves/protective clothing/eye protection/face protection.
If swallowed: Immediately call a poison center/doctor.
Specific treatment (see on this label).
Do NOT induce vomiting.
If on skin: Wash with plenty of water.
If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Take off contaminated clothing and wash it before reuse.

(Contd. on page 9)

Safety Data Sheet
acc. to OSHA HCS

Trade name: Peppermint (Mentha piperita)

(Contd. of page 8)

If skin irritation or rash occurs: Get medical advice/attention.
If eye irritation persists: Get medical advice/attention.
Wash contaminated clothing before reuse.
In case of fire: Use for extinction: CO2, powder or water spray.
Store in a well-ventilated place. Keep cool.
Store locked up.
Dispose of contents/container in accordance with local/regional/national/international regulations.
· **Chemical safety assessment:** *A Chemical Safety Assessment has not been carried out.*

16 Other information

This information is based on our present knowledge. However, this shall not constitute a guarantee for any specific product features and shall not establish a legally valid contractual relationship.

· **Department issuing SDS:** *Health Sciences and Product Safety*
· **Contact:** *Patricia Atkinson*
· **Date of preparation / last revision** *04/20/2018 / -*
· **Abbreviations and acronyms:**
ADR: Accord européen sur le transport des marchandises dangereuses par Route (European Agreement concerning the International Carriage of Dangerous Goods by Road)
IMDG: International Maritime Code for Dangerous Goods
DOT: US Department of Transportation
IATA: International Air Transport Association
ACGIH: American Conference of Governmental Industrial Hygienists
CAS: Chemical Abstracts Service (division of the American Chemical Society)
NFPA: National Fire Protection Association (USA)
HMIS: Hazardous Materials Identification System (USA)
VOC: Volatile Organic Compounds (USA, EU)
PBT: Persistent, Bioaccumulative and Toxic
vPvB: very Persistent and very Bioaccumulative
NIOSH: National Institute for Occupational Safety
OSHA: Occupational Safety & Health
TLV: Threshold Limit Value
PEL: Permissible Exposure Limit
REL: Recommended Exposure Limit
Flam. Liq. 4: Flammable liquids – Category 4
Skin Irrit. 2: Skin corrosion/irritation – Category 2
Eye Irrit. 2A: Serious eye damage/eye irritation – Category 2A
Skin Sens. 1: Skin sensitisation – Category 1
Asp. Tox. 1: Aspiration hazard – Category 1
· **** Data compared to the previous version altered.***