Toxicological profile for

Cedarwood 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*

§ IUPAC systematic name: Not applicable.

§ Synonyms:

8000-27-9: Caswell No. 165C; Cedar oil; Cedarwood Oil, Kenyan; Cedarwood oil; Cedarwood oil residues; Cedarwood oil, Virginia; Cedarwood oil, Chinese; Cedarwood oil, Virginiana; Cedarwood oil, decolorized; Cedrus atlantica oil; EPA Pesticide Chemical Code 040505; HSDB 1972; Oil cedar; Oil of cedar wood; Oil of cedarwood; Oils, cedarwood; Red cedarwood oil; UNII-PAD4FN7P2G; UNII-ZX5QRE4U60 (ChemIDplus).

68990-83-0: Cedarwood oil Texas; Juniperus deppeana wood oil; Texas cedarwood oil; UNII-4739QA5686; Oils, cedarwood, Texan (ChemIDplus)

85085-41-2: EINECS 285-370-3; Eastern Red Cedar; Juniperus virginiana extract; Juniper, Juniperus virginiana, ext.; Juniperus virginiana; Juniperus virginiana pollen; Juniperus virginiana twig (ChemIDplus)

8023-85-6: Cedarwood oil, Cedarwood oil Moroccan, Cedarwood oil, atlas (Cedrus atlantica); Cedrus atlantica oil; Oils, cedarwood, Atlas (ChemIDplus).

§ Gross formula: No data available to us at this time.

§ Structural formula: No data available to us at this time.

§ Molecular weight (g/mol): No data available to us at this time.

§ CAS registration number: 8000-27-9, 68990-83-0, 85085-41-2, 8023-85-6

§ Properties:

Melting point: 262.5°C (for CAS RNs 68990-83-0; 8000-27-9) (EPISuite).

Boiling point: 150-300°C (poorly defined material); 245.05°C (estimated) (for CAS RNs 68990-83-0; 8000-27-9) (EPISuite) 150-300°C (CAS RN 8000-27-9).

Solubility in water: 0.1504 mg/L at 25°C (estimated) (for CAS RNs 68990-83-0; 8000-27-9) (EPISuite)

pK_a: No data available to us at this time.

Flashpoint: >93.33°C (CIR, 2001).

Flammability limits (vol/vol%): No data available to us at this time.

(Auto)ignition temperature: No data available to us at this time.

Decomposition temperature: No data available to us at this time.

Stability: No data available to us at this time.

Vapour pressure: 0.007 mmHg at 20°C (CIR, 2001).

log K_{ow}: 4.8 (this value relates to Nexa Cedarwood Oil Moth Protection, containing 40% cedarwood oil) (US EPA, 2010); 5.74 (estimated) (for CAS RNs 68990-83-0; 8000-27-9) (EPISuite).

2. *General information*

2.1 Exposure

Cosmetics: No evidence (Cosmetics Bench Ref, 1996); but see below.
Food: Yes. As cedarwood oil alcohols and cedarwood oil terpenes (Burdock GA, 2010)
Environment: Yes (HSDB, 2003)
Pharmaceuticals: Yes (Martindale, 1993)

Cedarwood oil, Virginia (CAS RN 8000-27-9), cedarwood oil, Texas (CAS RN 68990-83-0) and cedarwood oil atlas (CAS RN 8023-85-6) were reported as used in fragrance compounds in 2011.

As taken from IFRA, 2011 available at http://www.ifraorg.org/en-us/ingredients#.WPi9gLtwYfk

“Both cedar oils are used mainly for perfuming soaps and other products, as well as a starting material for the isolation of cedrol (77-53-2).“

“The worldwide annual production amounts to 1500-200 t.”

As taken from Common Fragrance and Flavor Materials. Bauer K et al. 2001 Wiley-VCH Verlag GmbH. ISBN: 3-527-60020 (Electronic).

Cedrus atlantica bark oil (CAS RN 92201-55-3 / 8000-27-9) is used as a perfuming, masking and skin conditioning agent, Juniperus virginiana oil (CAS RN 8000-27-9 / 85085-41-2) as a masking and tonic agent, Juniperus Mexicana oil (CAS RN 68990-83-0) as a masking agent, Juniperus virginia wood extract (CAS RN 85085-41-2) as a perfuming and tonic agent and Juniperus virginia wood oil (CAS RN 85085-41-2) as a perfuming agent in cosmetics in the EU. As taken from CosIng (Cosmetic ingredients database). Available at http://ec.europa.eu/growth/tools-databases/cosing/, accessed March 2017.

Major uses:

IN FURNITURE POLISH [Arena, J.M. Poisoning: Toxicology-Symptoms Treatments. Third Edition. Springfield, Illinois: Charles C. Thomas, 1974., p. 199] **PEER REVIEWED**

FRAGRANCE INGREDIENT (MODIFIER OF OTHER OILS) IN SOAPS, IN PERFUMES; RAW MATERIAL FOR ALCOHOLS & TERPENES AS FLAVORINGS; INSECT REPELLENT; CLEARING AGENT IN MICROSCOPY; AGENT IN OIL IMMERSION MICROSCOPY [SRI] **PEER REVIEWED**

As insect repellant; the thickened oil is used in microscopy as a clearing agent and for use with immersion lenses. [Budavari, S. (ed.). The Merck Index - Encyclopedia of Chemicals, Drugs and Biologicals. Rahway, NJ: Merck and Co., Inc., 1989., p. 1073] **PEER REVIEWED**

In soap perfumes [Gerhartz, W. (exec ed.). Ullmann's Encyclopedia of Industrial Chemistry. 5th ed.Vol A1: Deerfield Beach, FL: VCH Publishers, 1985 to Present., p. VA11 219] **PEER REVIEWED**

Perfuming soap and other products, as a starting material for the isolation of cedrol...and other valuable fragrance materials. [Gerhartz, W. (exec ed.). Ullmann's Encyclopedia of Industrial Chemistry. 5th ed.Vol A1: Deerfield Beach, FL: VCH Publishers, 1985 to Present., p. VA11 219] **PEER REVIEWED**

To convey woody notes to fragrances and also of fixatives. [Kirk-Othmer Encyclopedia of Chemical Technology. 3rd ed., Volumes 1-26. New York, NY: John Wiley and Sons, 1978-1984., p. V16 322] **PEER REVIEWED**

As taken from HSDB, 2003 powered by TOXNET, 2017 available at http://toxnet.nlm.nih.gov/newtoxnet/hsdb.htm

Cedarwood oil (CAS RN 8000-27-9) is listed as an ingredient in inside the home, landscape/yard (1%), personal care, pesticides (2-4%) and pet care (2%) products and Juniperus Mexicana oil (CAS RN 68990-83-0) as an ingredient in inside the home (<1%), landscape/yard (3.1-6.875%), personal care (0.4%) and pet care (0.6%) products by the US Department of Health and Human Services (2016).

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%
alpha-cedrene	1459806867	16.30	1254215803	16.95
beta-funebrene	147086118	1.64	128617156	1.74
beta-cedrene	512708659	5.72	428339493	5.79
widdrene	1568662618	17.51	1356838927	18.34
beta-chamigrene	185416117	2.07	148548721	2.01
beta-himachelene	95075191	1.06	71292102	0.96
alpha-chamigrene + cedrene isomer	287779849	3.21	236796752	3.20
beta-himachalene isomer + α	127819733	1.43	104199319	1.41
cuparene	264155650	2.95	211264988	2.86
cedrol	1617263843	18.06	1362574845	18.42
widdrol	253971491	2.84	220367066	2.98
unknown	93455864	1.04	72242334	0.98
Total area %		73.83		75.63

2.3 Ingredient(s) from which it originates

“Cedarwood oils are extracted from several members of the family Cupressaceae, which includes true cedars, junipers, and cypresses. In the US, cedarwood oil is harvested mainly from Juniperus virginiana (Eastern red cedar or Virginia cedar)” (NTP, 2002).

“Virginia cedarwood oil is produced by steam distillation of sawdust, finely chipped waste wood from the manufacture of cedarwood products, or from stumps and logs of the red cedar; *Juniperus virginiana* L. (Cupressaceae). It is a light yellow to pale brown, viscous liquid with characteristic cedarwood odor.”

“While the Texas cedar grows in Mexico and other Central American countries, the Virginia cedar grows exclusively in the Southeast of the United States.”

As taken from Common Fragrance and Flavor Materials. Bauer K et al. 2001 Wiley-VCH Verlag GmbH. ISBN: 3-527-60020 (Electronic).

Cedrus atlantica bark oil (CAS RN 92201-55-3 / 8000-27-9) is the volatile oil obtained from the bark of Cedrus atlantica, Pinaceae.

Juniperus virginiana oil (CAS RN 8000-27-9 / 85085-41-2) is the volatile oil obtained from the fruits and leaves of the red cedar, Juniperus virginiana L., Cupressaceae.

Juniperus virginiana wood extract (CAS RN 85085-41-2) is an extract of the wood of the red cedar, Juniperus virginiana L., Cupressaceae.

Juniperus virginiana wood oil ("cedar wood oil Virginian"; CAS RN 85085-41-2) is an essential oil obtained from the wood and twigs of the red cedar, Juniperus virginiana L., Cupressaceae. It contains chiefly cedrene and cedral (cedar camphor).

Juniperus mexicana oil (CAS RN 68990-83-0) is the volatile oil obtained from Juniperus mexicana, Cupressaceae.

As taken from CosIng (Cosmetic ingredients database). Available at http://ec.europa.eu/growth/tools-databases/cosing/, accessed March 2017

3. *Status in legislation and other official guidance*

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

Cedarwood oil alcohols and cedarwood oil terpenes are approved for use in food in the USA (21CFR 172.515) and in the EU

Cosmetics (UK): not listed

Cedarwood oil, Texas cedarwood oil and oils, cedarwood, Atlas (CAS RNs 8000-27-9, 68990-83-0 and 8023-85-6, respectively) are listed in the US EPA Toxic Substances Control Act (TSCA) inventory and cedarwood oil and Texas cedarwood oil are 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 http://iaspub.epa.gov/sor_internet/registry/substreg/searchandretrieve/searchbylist/search.do

CAS RNs 8000-27-9, 68990-83-0 and 85085-41-2 are listed as fragrance ingredients by the US EPA Inert Finder Database (2017) and CAS RNs 8000-27-9, 68990-83-0 and 8023-85-6 by the International Fragrance Association (IFRA, 2011).

Pre-registered under REACH (“envisaged registration deadline 31 May 2018” for CAS RNs 8000-27-9 (oils, cedarwood), 68990-83-0 (oil, cedarwood, Texan) and 8023-85-6 (oils, cedarwood, Atlas); “envisaged registration deadline 30 November 2010” for CAS RN 85085-41-2 (Juniper, Juniperus virginiana, ext)) (ECHA, 2016a).

Cedarwood oil Himalayan (CAS RN 8000-27-9), cedarwood oil, Texas (CAS RN 68990-83-0), juniper, Juniperus virginiana, ext. (CAS RN 85085-41-2) and cedarwood oil, Atlas type (CAS RN 8023-85-6) are not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2016b)

Cedarwood oil (Juniperus virginiana) 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 and cigars of 0.15 % w/w tobacco and in pipe tobacco of 0.5%.

Oils, cedarwood (CAS RN 8000-27-9) are listed in the US EPA Inert Finder Database (2017) as approved for non-food use pesticide products.

4. *Metabolism/Pharmacokinetics*

4.1 Metabolism/metabolites

Housing animals using cedarwood bedding resulted in a highly significant reduction of hexobarbital sleeping time in C3H-A, CBA/J, and Swiss Albino mice, indicating induction of the enzymes responsible for hexobarbital oxidation. Using the same methodology, the authors demonstrated enzyme induction in CBA/J mice from Virginia cedarwood oil (Sabine, 1975). The increase in the duration of hexobarbital hypnosis following exposure of Swiss-Webster mice to cedar shavings was previously reported by Wade et al. These investigators then exposed mice to various fractions of cedarwood for up to 10 days and measured the duration of hexobarbital anesthesia, which suggested that cedrol and cedrene were the causative agents (Wade et al. 1968).

The effect of cedrene on hepatic metabolism was studied in Sprague-Dawley rats. Administration of cedrene using the oral, intraperitoneal and inhalation routes, increased the ethylmorphine N-demethylase activity and cytochrome P-450 content, while it had no effects on aniline hydroxylase activity (Hashimoto et al. 1972).

4.2 Absorption, distribution and excretion

No data available to us at this time.

4.3 Interactions

No data available to us at this time.

5. *Toxicity*

5.1 Single dose toxicity

Oral, rat: LD_{50}: > 5 g/kg bw (Moreno, 1974)
Skin, rabbit: LD_{50}: > 5 g/kg bw (Moreno, 1974)

5.2 Repeated dose toxicity

A 28-day inhalation study was performed in Sprague Dawley rats (5 male, 5 female) using Nexa Cedarwood Oil Moth Protection (40% cedarwood oil). Modified cages were covered by plastic panes (90%) and a single test substance blister pack, with dispenser volume 9 ml, was fastened. Levels of (-)-α-cedrene and (+)-cedrol, the test substance's main ingredients, were reported to be between 7 and 10 mg/100 L air for (-)-α-cedrene and 4.1 mg/100 L air for (+)-cedrol on the last day of the test. For the first 5 days, rats were observed to avoid the test substance. No other adverse effects or mortality occurred during the study and all animals remained at a normal weight (US EPA, 2010).

90-day topical studies have been performed in mice and rats but, although tables of results for individual animals for various endpoints can be downloaded from the website, no summary is available and the data are not presented in any easily-accessible format (NTP, 2005a).

“Virginia cedarwood oil is widely used as a fragrance material in household and personal products and as a naturally derived pesticide alternative. Due to conflicting literature on dermal exposures in animals and humans, concern for safe levels of human exposure remains. The present study evaluated the toxicity of cedarwood oil applied dermally to F344/N rats and B6C3F1/N mice for 13 weeks. Groups of 10 male and female rats and mice received no treatment (untreated control) or were administered cedarwood oil in 95% aqueous ethanol dermally at concentrations ranging from 0% (vehicle control), 6.25%, 12.5%, 25%, 50%, and 100% (undiluted). Rats and mice developed extensive skin lesions at the site of application. Benchmark dose modeling (BMD) was performed for the significantly increased skin lesions observed in the rat, to provide perspective for risk assessment applications. Benchmark dose modeling levels (BMDL) of 0.65 to 2.1% and 1.2 to 4.4% (equivalent to 13 to 42 mg/kg and 24 to 48 mg/kg, respectively) cedarwood oil were calculated for the most sensitive endpoint of epidermal hyperplasia in female rats and chronic active inflammation in male rats, respectively. These BMDL levels coincide with reported use levels in cosmetics and pesticides, raising the concern for human exposure.” As taken from Catlin NR et al. 2016. Food Chem. Toxicol. 98(Pt B), 159-168. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/?term=27769849

5.3 Reproduction toxicity

No good quality information in mammals was identified. Reviews state there are reports from the nineteenth century of abortion in women who ingested “large doses” of cedarwood oil (HSDB, 2003; NTP, 2002).

“Burkhart and Robinson (1978) described a high rate of rat pup deaths, which the authors felt was probably caused by Eastern cedarwood bedding, either through ingestion or inhalation of toxic compounds in the bedding or through the milk of the dams” (NTP, 2002). However it is unclear whether this was due to exposure of the parents (i.e. a reproductive or developmental effect) or to direct exposure of the offspring (i.e. normal toxicity).

“Beta-thujaplicin, a compound found in the heartwood of the western red cedar (Thuja plicata), was teratogenic when administered to ICR mice at very high doses. In vitro, beta-thujaplicin induced growth retardation and malformation of cultured embryos harvested at 9 days of gestation. In vivo, 420-1,000 mg/kg of beta-thujaplicin, given orally to pregnant ICR mice on day 9 of gestation, induced cleft palates and lips, facial dysmorphism, and other malformations at doses of 560 mg/kg or above in 18-d old fetuses… (Ogata *et al.*, 1999)” (NTP, 2002).

A developmental toxicity study with cedarwood oil has been conducted in chick embryos. Amniotic sacs of 3-day-old chick embryos were injected with an emulsion of cedarwood oil in saline (1 µl of a 1, 5 or 10% emulsion or 2 µl of a 10% emulsion) and a histopathological examination was carried out [extent of examination not clear from brief abstract]. Teratogenic effects were seen at 10% (abnormal wings at 1 µl; head at 2 µl; local effect at injection site) but no effects were seen at 5% and below (De Blasi & Schowing, 1988). [This seems a very low injection volume, and the relevance of this assay in chicks to mammals is uncertain.]

5.4 Mutagenicity

In a mouse micronucleus test, dermal application of cedarwood oil at up to 1290 mg/kg bw/day for 90 days produced no evidence of genotoxicity (NTP, 2005b).

No mutagenicity was seen in an Ames test with cedarwood oil at up to 333 µg/plate in *Salmonella typhimurium* strains TA98, TA100 and TA102, with or without S9 (NTP, 2007).

5.5 Cytotoxicity

No data available to us at this time.

5.6 Carcinogenicity

In an early study of tumour-promoting activity (Roe & Field, 1965), single dermal application of a known skin carcinogen (as initiator) to an unspecified number of mice, followed after 3 weeks by 33 weekly applications of "cedarwood oil" (sub-type unspecified) [presumably 0.25 ml/week], found no evidence of skin tumour promotion or epidermal hyperplasia (reported as "equivocal" evidence of epidermal hyperplasia in Salaman, 1961.)

"Cedarwood oil, most likely Virginia, has been reported to have tumor-producing properties on mouse skin" (Yeung & Foster, 2003).

American-born C3H-A and C3H-A fB mice raised in the US have nearly a 100% incidence of liver and mammary tumors. These strains, bred and reared in Australia on sawdust bedding from Douglas fir, had almost no spontaneous incidence of mammary and liver tumors, particularly after the first generation. In contrast, virtually all C3H-A mice reared in Australia but kept on US bedding (cedar) and fed US diets developed mammary tumors. The authors expressed their opinion that the cedar appeared to be the "carcinogenic" agent, noting that the results involved a limited number of animals (Sabine *et al.*, 1973).

"When … two groups of mice [were bedded] on either ¾ pine sawdust and ¼ cedar shavings or pine sawdust, both groups developed very high incidences of spontaneous mammary tumors and hepatomas. [The investigator] attributed the lower incidences of spontaneous tumors seen in the Australian study [Sabine *et al.*, 1973] to higher ectoparasite infestations and slightly lower growth rates (Heston, 1975)" (NTP, 2002).

"Vlahakis (1977) reported that the first generation of C3H-A^{vy}fB crossbred mice had the same high incidences of mammary and liver tumors whether they were raised using pine bedding or a mixture of pine plus red cedar shavings" (NTP, 2002).

"Cedrene prevented the butylated hydroxytoluene (BHT)-induced inhibition of lung tumors caused by intraperitoneal injection of urethan in strain A mice (Malkinson & Beer, 1984)" (NTP, 2002).

5.7 Irritation / immunotoxicity

Skin irritation was not observed in male and female volunteers patch-tested with cedarwood oil (24-72-hour closed patch tests), when tested in concentrations of 0.2% (148 volunteers), 2.0% (30 volunteers) and 20.0% (29 volunteers) (Fujii *et al*., 1972).

Roe and Field (1965) did not report any skin irritation when cedarwood oil was applied to the clipped, dorsal skin of 101 inbred mice (two doses of an unspecified amount, one week apart).

A 500mg 24h-exposure on rabbit skin produced a moderate irritant effect (RTECS, 2003).

Following tests, Cedarwood oil was shown not to be irritating (Urbach & Forbes, 1973; Kligman 1973).

Undiluted cedarwood oil Virginia applied to the backs of hairless mice was not irritating (HSDB, 2003; Opdyke, 1974).

Vasodilation was not seen following the application of undiluted Juniperus Virginiana Oil (2 ml of neat material or in ethanol) to the external ears of rabbits (Lacy *et al.*, 1987).

0.1 ml of undiluted Nexa Cedarwood Oil Moth Protection (containing 40% cedarwood) applied to the eyes of 3 albino rabbits led to the development of hyperemic blood vessels. This effect was reversible, and no lesions were observed on the cornea or iris. No other adverse effects were observed (US EPA, 2010).

Skin and respiratory tract sensitization

Cedarwood oil (Virginia and /or Texas) has been reported to have a slight local allergenic (acute and chronic) and acute local irritant properties (Sax, 1979). Dermatological data have indicated cedarwood oils to be generally nontoxic (Yeung & Foster, 2003).

“A maximization test was carried out on 25 volunteers. The material was tested at a concentration of 8% in petrolatum and produced no sensitization reactions (Kligman, 1973)” (Opdyke, 1974).

“Patch tests were used to investigate "cedar-poisoning" in 43 persons exposed to wood products or vegetation. The results obtained showed that the term “cedar poisoning” was a misnomer... (Tan & Mitchell, 1968)” (Opdyke, 1974).

An analysis of data from the Information Network of Departments of Dermatology (IVDK) looked at data from 15682 patients who had been patch tested with at least one essential oil. 6233 patients were patch tested with 10% cedarwood oil in petrolatum. Of those tested, following age and sex standardisation, 0.83% gave a positive result for sensitization (Uter et al., 2010).

A maximization test carried out on 10 guinea pigs using Nexa Cedarwood Oil Moth Protection (containing 40% cedarwood oil) showed no positive sensitization reactions (US EPA, 2010).

A woman who suffered a reaction to a temporary henna tattoo, showed a strongly positive response to cedarwood oil (10% in petrolatum) at 1, 2 and 3 days after patch testing (Temesvari et al., 2002).

No sensitization reactions were reported on day 3 or 4 in patch tests on 95 contact dermatitis patients with cedarwood oil at 1 or 5% in petrolatum (Frosch et al., 1995).

A 66-year old woman exhibiting contact dermatitis following the use of Vicks VapoRub [containing cedar leaf oil, CAS: 8007-20-3] was patch tested for a range of different allergens, including cedarwood oil. A weak positive reaction (+) was observed for cedarwood oil, among others (Noiles and Pratt, 2010).

A number of other papers indexed in TRACE report skin sensitization studies with various cedarwood oils.

“The diagnostic workup of contact allergy to fragrances must not be limited to patch testing with the two well-established fragrance mixes. False-positive reactions to these mixes occur in up to

50 % of the patch tested patients. For the diagnostic work-up of positive reactions, and in cases of suspected fragrance allergy, patch testing with the single mix components and additional fragrances is mandatory. Frequently sensitizing fragrance materials are the 14 components of the two fragrance mixes and tree moss (Evernia furfuracea), ylang ylang oil (I + II; Cananga odorata), lemongrass oil (Cymbopogon schoenanthus), sandalwood oil (Santalum album), jasmine absolute (Jasminum spp.), and, less frequently, clove oil (Eugenia caryophyllus), cedarwood oil (Cedrus atlantica/deodara, Juniperus virginiana), Neroli oil (Citrus aurantium amara flower oil), salicylaldehyde, narcissus absolute (Narcissus spp.), and patchouli oil (Pogostemon cablin)." As taken from Geier J and Uter W. 2015. Hautarzt. 66(9), 674-9. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26253114

"Acne vulgaris is a widely prevalent chronic skin disease. Although multiple treatments are available, acne can sometimes be refractory to these treatments. The use of alternative medical therapies has increased within dermatology and for acne. This case report describes a patient in whom the addition of cedarwood oil was helpful in controlling acne." As taken from Hassoun LA et al. 2016. J. Altern. Complement. Med. 22(3), 252-3. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26910133

"Virginia cedarwood oil is widely used as a fragrance material in household and personal products and as a naturally derived pesticide alternative. Due to conflicting literature on dermal exposures in animals and humans, concern for safe levels of human exposure remains. The present study evaluated the toxicity of cedarwood oil applied dermally to F344/N rats and B6C3F1/N mice for 13 weeks. Groups of 10 male and female rats and mice received no treatment (untreated control) or were administered cedarwood oil in 95% aqueous ethanol dermally at concentrations ranging from 0% (vehicle control), 6.25%, 12.5%, 25%, 50%, and 100% (undiluted). Rats and mice developed extensive skin lesions at the site of application. Benchmark dose modeling (BMD) was performed for the significantly increased skin lesions observed in the rat, to provide perspective for risk assessment applications. Benchmark dose modeling levels (BMDL) of 0.65 to 2.1% and 1.2 to 4.4% (equivalent to 13 to 42 mg/kg and 24 to 48 mg/kg, respectively) cedarwood oil were calculated for the most sensitive endpoint of epidermal hyperplasia in female rats and chronic active inflammation in male rats, respectively. These BMDL levels coincide with reported use levels in cosmetics and pesticides, raising the concern for human exposure." As taken from Catlin NR et al. 2016. Food Chem. Toxicol. 98(Pt B), 159-168. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/?term=27769849

5.8 All other relevant types of toxicity

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

Endpoint	Tested level (ppm)	Reference
In vitro genotoxicity	27	JTI KB Study Report(s)
In vitro cytotoxicity	27	JTI KB Study Report(s)

A positive phototoxic reaction (skin erythema [redness]) was induced in pigs and mice by exposure to UV light after a previous application of cedarleaf oil, cedarwood oil atlas, cedarwood oil (Texas) or cedarwood Viginia (Forbes *et al.*, 1977).

No photoxic effects were reported for undiluted cedarwood oil Texas on hairless mice and swine (Urbach & Forbes, 1975).

6. *Functional effects on:*

6.1 Broncho/pulmonary system

A 28-day inhalation study was performed on Sprague-Dawley rats (5 male, 5 female) using Nexa Cedarwood Oil Moth Protection (40% cedarwood oil). Modified cages were covered by plastic panes (90%) and a single test substance blister pack, with dispenser volume 9 ml, was fastened. Levels of (-)-α-cedrene and (+)-cedrol, the chemical's main ingredients, were reported to be between 7 and 10 mg/100L air for (-)-α-cedrene and 4.1 mg/100L air for (+)-cedrol on the last day of the test. For the first 5 days, rats were observed to avoid the test substance. No other adverse effects or mortality occurred during the study and all animals remained at a normal weight (US EPA, 2010).

6.2 Cardiovascular system

It is well known that odors affect behaviors and autonomic functions. Previous studies reported that some compounds in cedar wood essence induced behavioral changes including sedative effects. In the present study, we analyzed cardiovascular and respiratory functions while subjects were inhaling fumes of pure compound (Cedrol) which was extracted from cedar wood oil.Vaporized Cedrol (14.2+/-1.7 microg/l, 5 l/min) and blank air (5 l/min) were presented to healthy human subjects (n=26) via a face mask, while ECGs, heart rate (HR), systolic blood pressure (SBP), diastolic BP (DBP), and respiratory rates (RR) were monitored. Statistical analyses indicated that exposure to Cedrol significantly decreased HR, SBP, and DBP compared to blank air while it increased baroreceptor sensitivity. Furthermore, respiratory rate was reduced during exposure to Cedrol. These results, along with the previous studies reporting close relationship between respiratory and cardiovascular functions, suggest that these changes in respiratory functions were consistent with above cardiovascular alterations (Dayawansa *et al*., 2003).

6.3 Nervous system

It has been reported that cedarwood oil has sedative effects when inhaled. In this study, the sedative effects of inhaled cedrol, which is a major component of cedarwood oil have been examined. Accumulative spontaneous motor activity was significantly decreased in the cedrol-exposed Wistar rats. Similar results were confirmed in caffeine-treated Wistar rats, spontaneously hypertensive rats (SHR), and ddY mice. In addition, exposure to cedrol prolonged pentobarbital-induced sleeping time in Wistar rats. To investigate whether cedrol, which has a very faint aroma, affects the olfactory system, the nasal cavities of Wistar rats were treated with zinc sulfate to reduce olfactory function. Two days later, the pentobarbital-induced sleep time was measured as described above. Compared to intact rats, the sleep prolongation effect was decreased in a lavender-roman chamomile mixed oil exposure positive control group, indicating that olfactory function was impaired. In contrast, prolongation of the sleeping time did not change in the cedrol exposure group. The above findings indicate that cedrol inhalation had marked sedative effects regardless of the animal species or the functional state of the autonomic nerves, suggesting that the mechanism of action is via a pathway other than the olfactory system (Kagawa *et al*., 2003).

“Several plant-derived essential oils have been known for over a century to have epileptogenic properties. We report three healthy patients, two adults and one child, who suffered from an isolated generalized tonic-clonic seizure and a generalized tonic status, respectively, related to the absorption of several of these oils for therapeutic purposes. No other cause of epilepsy was found, and outcome was good in the two adult cases, but the course has been less favorable in the child. A survey of the literature shows essential oils of 11 plants to be powerful convulsants (eucalyptus, fennel, hyssop, pennyroyal, rosemary, sage, savin, tansy, thuja, turpentine, and wormwood) due to their content of highly reactive monoterpene ketones, such as camphor, pinocamphone, thujone, cineole, pulegone, sabinylacetate, and fenchone. Our three cases strongly support the concept of plant-related toxic seizure. Nowadays the wide use of these compounds in certain unconventional medicines makes this severe complication again possible” (Burkhard et al. 1999).

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

Housing animals using cedarwood bedding resulted in a highly significant reduction of hexobarbital sleeping time in C3H-A, CBA/J, and Swiss Albino mice, indicating induction of the enzymes responsible for hexobarbital oxidation. Using the same methodology, the authors demonstrated enzyme induction in CBA/J mice from Virginia cedarwood oil (Sabine, 1975). The increase in the duration of hexobarbital hypnosis following exposure of Swiss-Webster mice to cedar shavings was previously reported by Wade et al. These investigators then exposed mice to various fractions of cedarwood for up to 10 days and measured the duration of hexobarbital anesthesia, which suggested that cedrol and cedrene were the causative agents (Wade et al., 1968).

“The effect of cedrene on in vitro hepatic metabolism was studied in Sprague-Dawley rats. Administration of cedrene using the oral, intraperitoneal and inhalation routes, increased the ethylmorphine N-demethylase activity and cytochrome P-450 content, while it had no effects on aniline hydroxylase activity (Hashimoto *et al.*, 1972)” (NTP, 2002).

“Medicinal plants are a rich source of ligands for nuclear receptors. The present study was aimed to screen a collection of plant extracts for PPARα/γ-activating properties and identify the active extract that can stimulate cellular glucose uptake without enhancing the adipogenesis. A reporter gene assay was performed to screen ethanolic extracts of 263 plant species, belonging to 94 families, for activation of PPARα and PPARγ. Eight extracts showed activation of PPARγ, while 22 extracts showed activation of PPARα. The extracts of five plants (Daphne gnidium, Illicium anisatum, Juniperus virginiana, Terminalia chebula, and Thymelaea hirsuta) showed activation of both PPARα and PPARγ and out of them, D. gnidium and T. hirsuta markedly increased PPARα/γ protein expression. All five extracts showed an increase in cellular glucose uptake. Of the five dual agonists, T. chebula and T. hirsuta did not show any increase in differentiation of 3T3-L1 preadipocytes, but I. anisatum caused an increase in adipogenesis, while D. gnidium and J. virginiana were toxic to adipocytes. The adipogenic effect of rosiglitazone was antagonized by T. chebula and T. hirsuta. It was concluded that T. hirsuta and T. chebula retain the property of elevating glucose uptake as PPARα/γ dual agonists without the undesired side effect of adipogenesis. This is the first report to reveal the PPARα/γ dual agonistic action and glucose uptake enhancing property of T. hirsuta and T. chebula.” As taken from Yang MH et al. 2013. Planta Med. 79(12), 1084-95. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23877921

"Virginia cedarwood oil is widely used as a fragrance material in household and personal products and as a naturally derived pesticide alternative. Due to conflicting literature on dermal exposures in animals and humans, concern for safe levels of human exposure remains. The present study evaluated the toxicity of cedarwood oil applied dermally to F344/N rats and B6C3F1/N mice for 13 weeks. Groups of 10 male and female rats and mice received no treatment (untreated control) or were administered cedarwood oil in 95% aqueous ethanol dermally at concentrations ranging from 0% (vehicle control), 6.25%, 12.5%, 25%, 50%, and 100% (undiluted). Rats and mice developed extensive skin lesions at the site of application. Benchmark dose modeling (BMD) was performed for the significantly increased skin lesions observed in the rat, to provide perspective for risk assessment applications. Benchmark dose modeling levels (BMDL) of 0.65 to 2.1% and 1.2 to 4.4% (equivalent to 13 to 42 mg/kg and 24 to 48 mg/kg, respectively) cedarwood oil were calculated for the most sensitive endpoint of epidermal hyperplasia in female rats and chronic active inflammation in male rats, respectively. These BMDL levels coincide with reported use levels in cosmetics and pesticides, raising the concern for human exposure." As taken from Catlin NR et al. 2016. Food Chem. Toxicol. 98(Pt B), 159-168. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/?term=27769849

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 Cedarwood oil and an additive free, reference cigarettes were tested in a battery of *in vitro* and/or *in vivo* test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the condensate was not changed by the addition of Cedarwood oil. Table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
Smoke chemistry	65 (CAS 85085-29-6)	JTI KB Study Report(s)
	3	Roemer et al., 2014
In vitro genotoxicity	26 (CAS 8000-27-9)	Renne et al., 2006
	60 (CAS 8023-85-6)	fGLH Study Report (2010)
	3	Roemer et al., 2014

In vitro cytotoxicity	65 (CAS 85085-29-6)	JTI KB Study Report(s)
	60 (CAS 8023-85-6)	fGLH Study Report (2010)
	3	Roemer et al., 2014
Inhalation study	26 (CAS 8000-27-9)	Renne et al., 2006
	65 (CAS 85085-29-6)	JTI KB Study Report(s)
	3	Schramke et al., 2014
Skin painting	65 (CAS 85085-29-6)	JTI KB Study Report(s)
In vivo genotoxicity	3	Schramke et al., 2014

9. *Ecotoxicity*

9.1 Environmental fate

EPISuite provides the following data for CAS RNs 68990-83-0; 8000-27-9:

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

Bond Method :	1.95E-001 atm-m3/mole (1.97E+004 Pa-m3/mole)
Group Method:	Incomplete
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:	HLC: 2.467E-004 atm-m3/mole (2.500E+001 Pa-m3/mole) VP: 0.000138 mm Hg (source: MPBPVP) WS: 0.15 mg/L (source: WSKOWWIN)

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

Log Kow used:	5.74 (KowWin est)
Log Kaw used:	0.902 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate):	4.838
Log Koa (experimental database):	None

Probability of Rapid Biodegradation (BIOWIN v4.10):

Biowin1 (Linear Model): Biowin2 (Non-Linear Model) : Biowin3 (Ultimate Survey Model): Biowin4 (Primary Survey Model) : Biowin5 (MITI Linear Model) : Biowin6 (MITI Non-Linear Model): Biowin7 (Anaerobic Linear Model):	0.2824 0.0342 2.3233 (weeks-months) 3.2460 (weeks) 0.3635 0.1288 -0.5859
Ready Biodegradability Prediction:	NO

Hydrocarbon Biodegradation (BioHCwin v1.01):

LOG BioHC Half-Life (days) :	3.2002
BioHC Half-Life (days) :	1585.7919

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

Vapor pressure (liquid/subcooled):	7.33 Pa (0.055 mm Hg)
Log Koa (Koawin est):	4.838
Kp (particle/gas partition coef. (m3/ug)): Mackay model: Octanol/air (Koa) model:	 4.09E-007 1.69E-008

Fraction sorbed to airborne particulates (phi):

Junge-Pankow model:	1.48E-005
Mackay model:	3.27E-005
Octanol/air (Koa) model:	1.35E-006

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

Hydroxyl Radicals Reaction:

OVERALL OH Rate Constant =	96.6352 E-12 cm3/molecule-sec
Half-Life =	0.111 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life =	1.328 Hrs

Ozone Reaction:

OVERALL Ozone Rate Constant =	43.000000 E-17 cm3/molecule-sec
Half-Life =	0.027 Days (at 7E11 mol/cm3)
Half-Life =	38.378 Min
Reaction With Nitrate Radicals May Be Important!	
Fraction sorbed to airborne particulates (phi):	2.38E-005 (Junge-Pankow, Mackay avg) 1.35E-006 (Koa method)
Note: the sorbed fraction may be resistant to atmospheric oxidation	

Soil Adsorption Coefficient (KOCWIN v2.00):

Koc :	1.738E+004 L/kg (MCI method)
Log Koc:	4.240 (MCI method)
Koc :	9.58E+004 L/kg (Kow method)
Log Koc:	4.981 (Kow method)

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]:

Rate constants can NOT be estimated for this structure!

Volatilization from Water:

Henry LC: 0.195 atm-m3/mole (estimated by Bond SAR Method)

Half-Life from Model River:	1.463 hours
Half-Life from Model Lake:	135.8 hours (5.66 days)

Removal In Wastewater Treatment (recommended maximum 95%):

Total removal:	98.67 percent
Total biodegradation:	0.25 percent
Total sludge adsorption:	61.90 percent
Total to Air:	36.52 percent

(using 10000 hr Bio P,A,S)

Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	1	2.76	1000
Water	25.8	900	1000
Soil	48	1.8e+003	1000
Sediment	25.2	8.1e+003	0

Persistence Time: 305 hr

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that oils, cedarwood (CAS RN 8000-27-9) and oils, cedarwood, Texan (CAS RN 68990-83-0) are not persistent in the environment.
Data accessed March 2017 on the OECD website: http://webnet.oecd.org/CCRWeb/Search.aspx

9.2 Aquatic toxicity

Aedes aegypti (Yellow Fever Mosquito) larvae, 24-hr LC_{50}: <10 mg/L (Amer and Mehlhorn, 2006).
Aedes aegypti (Yellow Fever Mosquito) larvae, 12-hr LC_{100}: 50 mg/L (Amer and Mehlhorn, 2006).
Anopheles stephensi (Mosquito) larvae, 24-hr LC_{50}: ~10 mg/L (Amer and Mehlhorn, 2006).
Culex quinquefasciatus (Southern House Mosquito) larvae, 24-hr LC_{50}: ~10 mg/L (Amer and Mehlhorn, 2006).
Bulinus truncatus (Snail), 24-hr LC_{50}: 470 (380-580) µg/L (Lahlou, 2003).
Bulinus truncatus (Snail), 24-hr LC_{90}: 690 (530-900) µg/L (Lahlou, 2003).

The Ecological Categorization Results from the Canadian Domestic Substances List state that oils, cedarwood (CAS RN 8000-27-9) and oils, cedarwood, Texan (CAS RN 68990-83-0) are inherently toxic to aquatic organisms:

	68990-83-0	**8000-27-9**
Pivotal value for iT (mg/l)	0.05	0.05
Comment iT	Group: oil; Subgroup: Cedarwood oil	Group: oil; Subgroup: Cedarwood oil
Toxicity to fish (LC50 in mg/l) as predicted by Oasis Forecast M v1.10	0.1044	0.1046
Toxicity to fish (LC50 in mg/l) as predicted by PNN	11.17487	11.17487
Toxicity to daphnia (EC50 in mg/l) as predicted by Topkat v6.1	48.3	48.3
Toxicity to fish, daphnia, algae or mysid shrimp (EC50 or LC50 in mg/l) as predicted by Ecosar v0.99g	0.05	0.05
Chronic toxicity to daphnia or algae (EC50 in mg/l) as predicted by Ecosar v0.99g	0.017	0.017

Toxicity to fish (LC50 in mg/l) as predicted by Neutral Organics QSAR in Ecosar v0.99g	1.52E-001	1.52E-001

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

Record for cedarwood oils (CAS RN 8000-27-9):

Spec. Sci. Name **Spec. Common Name**	**Exp. Type** **Chem. Anal.**	**Media Type** **Loc**	**Resp. Site** **Obs. Dur. (Days)**	**Endpoint** **BCF**	**Trend** **Eff%**	**Effect** **Effect Meas.**	**Conc (Standardized)** **Appl. Rate**
Insects/Spiders							
Aedes aegypti Yellow Fever Mosquito	S U	FW LAB	1	LC50	INC	MOR MORT	F < 10000 ug/L
Anopheles stephensi Mosquito	S U	FW LAB	1	LC50	INC	MOR MORT	F ~ 10000 ug/L
Culex quinquefasciatus Southern House Mosquito	S U	FW LAB	1	LC50	INC	MOR MORT	F ~ 10000 ug/L
Aedes aegypti Yellow Fever Mosquito	S U	FW LAB	0.5	NR-LETH	INC 100	MOR MORT	F 50000 ug/L
Molluscs							
Bulinus truncatus Snail	S U	FW LAB	1	LC50	INC	MOR MORT	F 470 (380-580) ug/L
Bulinus truncatus Snail	S U	FW LAB	1	LC90	INC	MOR MORT	F 690 (530-900) ug/L

As taken from the EPA ECOTOX Database, accessed March 2017 available at http://cfpub.epa.gov/ecotox/quick_query.htm

ECOSAR Version 1.11 provides the following aquatic toxicity data for CAS RNs 8000-27-9 and 68990-83-0:

Values used to Generate ECOSAR Profile
Log Kow: 5.743 (EPISuite Kowwin v1.68 Estimate)
Wat Sol: 0.001669 (mg/L, EPISuite WSKowwin v1.43 Estimate)

ECOSAR v1.11 Class-specific Estimations
Neutral Organics

ECOSAR Class		**Organism**	**Duration**	**End Pt**	**Predicted mg/L (ppm)**
Neutral Organics	:	Fish	96-hr	LC50	0.073 *
Neutral Organics	:	Daphnid	48-hr	LC50	0.059 *
Neutral Organics	:	Green Algae	96-hr	EC50	0.182 *
Neutral Organics	:	Fish		ChV	0.011 *
Neutral Organics	:	Daphnid		ChV	0.015 *
Neutral Organics	:	Green Algae		ChV	0.103 *
Neutral Organics	:	Fish (SW)	96-hr	LC50	0.094 *

Neutral Organics	:	Mysid	96-hr	LC50	0.006 *
Neutral Organics	:	Fish (SW)		ChV	0.100 *
Neutral Organics	:	Mysid (SW)		ChV	0.000158

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

Class Specific LogKow Cut-Offs
If the log Kow of the chemical is greater than the endpoint specific cut-offs presented below, then no effects at saturation are expected for those endpoints.

Neutral Organics:
Maximum LogKow: 5.0 (Fish 96-hr LC50; Daphnid LC50, Mysid LC50)

9.3 Sediment toxicity

No data available to us at this time

9.4 Terrestrial toxicity

Apis mellifera (Honey Bee), 4-hr NOAEL: 75 ml/L (Mayer et al. 2001).
Formica aerata (Grey Field Ant), 2-day NOAEL: 2 g/60 cm (Shorey et al. 1993).

“In laboratory studies, the Argentine ant, Linepithema humile (Mayr), and the odorous house ant, Tapinoma sessile (Say), avoided aromatic cedar mulch as a nesting substrate. Both ant species were killed when confined with fresh aromatic cedar mulch in sealed containers. However, when confined with cedar mulch that had been aged outdoors for up to 140 d, mortality of L. humile was complete regardless of mulch age, whereas T. sessile mortality declined significantly over the mulch-aging period. Argentine ant susceptibility to aromatic cedar mulch was also greater than that of the odorous house ant when colonies were restricted to mulch in open trays. In addition, commercial aromatic cedar oil was lethal to both ant species. Our results suggest that aromatic cedar mulch may serve as an effective component of a comprehensive urban ant management program” (Meissner & Silverman 2001).

“Cercariae of Schistosoma mansoni exposed to cedarwood oil show early phases of the penetration response before they succumb to the toxic effects of the oil. The toxic effect is also seen when cercariae are exposed to certain components of the oil followed by exposure to a known penetration stimulant, linolenic acid, which accelerates the inactivation of the organism. It is postulated that the process of penetration which results in the disruption of the cercarial glycocalyx alters physiological processes related to osmoregulation. This may increase the absorption of the toxic substances in cedarwood oil by the organisms” (Naples et al. 1992).

“Virginia cedarwood oil (3%), cedrene (2%), and cedrol (2%) were all highly toxic to Peanut Trash Bug colonies. Cedarwood oil and cedrene also affected the reproductive behavior of adults or hatchability of eggs. Colonies of German cockroaches (Blatella germanica) were not affected by cedarwood from Juniperus virginiana” (Sabine, 1975).

“Cedar and some of its derivatives (oil of cedarwood) disrupted the reproductive and development cycle of a number of insects, incl the peanut trash bug, the Indian meal moth and the forage mite” (Sabine, 1975).

Record for cedarwood oils (CAS RN 8000-27-9):

Spec. Sci. Name Spec. Common Name	Resp. Type Exp. Dur. (Days)	Media Type Test Loc.	Exp. Site Chem. Anal.	Dose# Res. Sample Unit	Endpoint BAF/BCF	Effect Effect Meas.
Insects/Spiders						
Formica aerata Grey Field Ant	 2	LIT FIELDN	EN U	2	NOEL	AVO CHEM
Insects/Spiders; Standard Test Species						
Apis mellifera Honey Bee	 0.167	NONE FIELDN	HS U	2	NOEL	AVO CHEM
Apis mellifera Honey Bee	 0.042	NONE FIELDN	HS U	2	NOEL	AVO CHEM
Reptiles						
Boiga irregularis Brown Tree Snake	 0.004	NONE LAB	HS U	3		BEH NMVM/
Boiga irregularis Brown Tree Snake	 5	NONE LAB	HS U	3	NR-ZERO	MOR MORT

As taken from the EPA ECOTOX Database, accessed March 2017 available at http://cfpub.epa.gov/ecotox/quick_query.htm

"Heartwood samples from Juniperus virginiana L. were extracted with liquid carbon dioxide, and the bioactivity of carbon dioxide-derived cedarwood oil (CWO) toward several species of ants and cedrol toward ticks was determined. Repellency was tested for ants, and toxicity was tested for ticks. Ants in an outdoor bioassay were significantly repelled by the presence of CWO on a pole leading to a sugar-water solution. Similarly, CWO was a significant repellent barrier to red imported fire ants and prevented them from finding a typical food source. Black-legged tick nymphs exhibited dosage-dependent mortality when exposed to cedrol and at the highest dosage (i.e., 6.3 mg/ml) tested, the cedrol killed 100% of the ticks. These repellency and toxicity results together demonstrate a clear potential for the use of CWO as a pest control agent." As taken from Eller FJ et al. 2014. Environ. Entomol. 43, 762-766. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/24690252

ECOSAR Version 1.11 provides the following terrestrial toxicity data for CAS RNs 8000-27-9 and 68990-83-0:

Values used to Generate ECOSAR Profile
Log Kow: 5.743 (EPISuite Kowwin v1.68 Estimate)
Wat Sol: 0.001669 (mg/L, EPISuite WSKowwin v1.43 Estimate)

ECOSAR v1.11 Class-specific Estimations
Neutral Organics

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)

Neutral Organics :	Earthworm	14-day	LC50	145.363 *

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

9.5 All other relevant types of ecotoxicity

EPISuite provides the following data for CAS RNs 68990-83-0; 8000-27-9:

Bioaccumulation Estimates (BCFBAF v3.01):

Log BCF from regression-based method:	3.456 (BCF = 2859 L/kg wet-wt)
Log Biotransformation Half-life (HL):	1.2568 days (HL = 18.06 days)
Log BCF Arnot-Gobas method (upper trophic):	3.704 (BCF = 5058)
Log BAF Arnot-Gobas method (upper trophic):	4.499 (BAF = 3.153e+004)
log Kow used:	5.74 (estimated)

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that oils, cedarwood (CAS RN 8000-27-9) and oils, cedarwood, Texan (CAS RN 68990-83-0) are bioaccumulative in the environment.
Data accessed March 2017 on the OECD website: http://webnet.oecd.org/CCRWeb/Search.aspx

10. *References*

§ Amer A and Mehlhorn H (2006). Larvicidal Effects of Various Essential Oils Against Aedes, Anopheles, and Culex Larvae (Diptera, Culicidae). Parasitol. Res. 99(4): 466-472 (cited in US EPA, 2012).

§ Bauer K et al. (2001). Common Fragrance and Flavor Materials. 2001 Wiley-VCH Verlag GmbH. ISBN: 3-527-60020 (Electronic).

§ Burdock GA (2010). Fenaroli's Handbook of Flavor Ingredients. Sixth Edition. CRC Press. ISBN 0-8493-0946-8.

§ Burkhart CA & Robinson JL (1978). High rat pup mortality attributed to the use of cedar-wood shavings as bedding. Lab. Animals, 12, 221-222 (cited in NTP, 2002).

§ Burkhard PR et al. (1999). Plant-induced seizures: Reappearance of an old problem. Journal of Neurology 246(8), 667-670 (Toxline abstract).

§ Catlin NR et al. (2016). Dose-response assessment of the dermal toxicity of Virginia cedarwood oil in F344/N rats and B6C3F1/N mice. Food Chem. Toxicol. 98(Pt B), 159-168. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/?term=27769849

§ ChemIDplus. Accessed March 2017. Available at https://chem.nlm.nih.gov/chemidplus/

§ CIR (2001). Cosmetic Ingredient Review. Final report on the safety assessment of Juniperus Communis extract, Juniperus Oxycedrus extract, Juniperus Oxycedrus Tar, Juniperus Phoenicea extract, and Juniperus Virginiana extract. Int. J. Toxic. 20 41-56.

§ CosIng (undated). Cosmetic ingredients database. Records for CAS RNs 8000-27-9, 68990-83-0 and 85085-41-2. Accessed March 2017. Available at http://ec.europa.eu/growth/tools-databases/cosing/

- Cosmetics Bench Reference (1996). Published by Cosmetics and Toiletries. ISBN 0-931710-51-0.
- Dayawansa S et al. (2003). Autonomic responses during inhalation of natural fragrance of Cedrol in humans. Auton Neurosci. 108(1-2):79-86). PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.cgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=14614968&query_hl=27&itool=pubmed_docsum
- De Blasi and Schowing J (1988). Study of the teratogenicity of essential oils. Effect of oil of thuja on the development of the chick embryo. Teratology, 38(2), 16A [Abstract].
- 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
- 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
- ECHA (2016a). European Chemicals Agency. Information on Chemicals. Records for oils, cedarwood; oils, cedarwood, Texan, juniper, Juniperus virginiana, ext. and oils, cedarwood, Atlas. Last updated 10 May 2016. Available at: https://echa.europa.eu/information-on-chemicals/pre-registered-substances
- ECHA (2016b). European Chemicals Agency. Classification and Labelling (C&L) Inventory database. Last updated 2 December 2016. Available at: http://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database
- ECOSAR. Records for oils, cedarwood (CAS RN 8000-27-9) and oils, cedarwood, Texan (CAS RN 68990-83-0). Accessed March 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
- Eller FJ et al. (2014). Bioactivity of cedarwood oil and cedrol against arthropod pests. Environ. Entomol. 43, 762-766. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/24690252
- EPA ECOTOX Database. Record for cedarwood oils. Accessed March 2017. Available at: http://cfpub.epa.gov/ecotox/quick_query.htm
- EPISuite (undated). Records for oils, cedarwood (CAS RN 8000-27-9) and oils, cedarwood, Texan (CAS RN 68990-83-0). Accessed March 2017. (EPISuite content has not been updated since 2012, version 4.11.) EPISuite is available to download at https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface
- fGLH Study Report (2010)
- Forbes P D *et al.* (1977). Phototoxicity testing of fragrance raw materials. Food and Cosmetic Toxicology, 15, 55-60.
- Frosch P J *et al.* (1995). 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, 33, 333-342.
- Fujii T *et al.* (1972). Studies on compounded perfumes for toilet goods. On the non-irritative compounded perfumes for soaps. Yukugaku 21, 904-908 (cited in CIR, 2001).

§ Geier J and Uter W (2015). Diagnostic workup of fragrance allergy. [Article in German.] Hautarzt. 66(9), 674-9. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26253114

§ Hashimoto et al. 1972

§ Hassoun LA et al. (2016). Cedarwood Oil as Complementary Treatment in Refractory Acne. J. Altern. Complement. Med. 22(3), 252-3. PubMed, 2017 available at http://www.ncbi.nlm.nih.gov/pubmed/26910133

§ Heston WE (1975). Testing for possible effects of cedar wood shavings and diet on occurrence of mammary gland tumors and hepatomas in C3H-A-vy and C3H-Avy-fB mice. J. Natl Cancer Inst. 54(4): 1011-4.

§ HSDB (2003). Record for cedarwood oil. Hazardous Substances Databank Number: 1972. Last Revision Date: 14 February 2003. Accessed March 2017. Available at http://toxnet.nlm.nih.gov/newtoxnet/hsdb.htm

§ IFRA (2011). International Fragrance Association ingredients list. Last updated in 2011. Accessed March 2017. Available at http://www.ifraorg.org/en-us/ingredients#.WPi9gLtwYfk

§ JTI KB Study Report(s)

§ JTI Study Report (s).

§ Kagawa D et al. (2003). The sedative effects and mechanism of action of cedrol inhalation with behavioral pharmacological evaluation. Planta Med. 2003 Jul;69(7):637-41.

§ Kligman A M (1974). Report to RIFM, 31 October (cited in Opdyke, 1974).

§ Lacy MJ *et al.* (1987). D-Limonene: an effective vasodilator for use in collecting rabbit blood. Lab Anim Sci37, 485-487 (cited in CIR, 2001).

§ Lahlou M (2003). Composition and Molluscicidal Properties of Essential Oils of Five Moroccan Pinaceae. Pharmaceut. Biol.41(3): 207-210 (cited in US EPA, 2012).

§ Levenstein I (1975). Report to RIFM, 30 January (cited in Opdyke, 1976).

§ Lloyd R A et al (1976). Flue-cured tobacco flavour. 1. Essence and essential oil components. Tobacco Science, 20, 40-48.

§ Malkinson AM & Beer DS (1984). Pharmacologic and genetic studies on the modulatory effects of butylated hydroxytoluene on mouse lung adenoma formation. J. Natl Cancer Inst. 73(4): 925-33.

§ Martindale (1993). The Extra Pharmacopoeia. Edited by J E F Reynolds. Thirteenth edition. The Pharmaceutical Press. ISBN 0-85369-300-5.

§ Mayer DF et al. (2001). Field Evaluation of Non-Pesticide Chemicals as Honey Bee Repellents. In: L.P. Elzunces, C.Elissier, & G.B.Lewis (Eds.), Hazards of Pesticides to Bees, Institute National de la Recherche Agronomique, Paris, France:159-168 (cited in US EPA, 2012).

§ Meissner HE & Silverman J (2001). Effects of aromatic cedar mulch on the Argentine ant and the odorous house ant (Hymenoptera: Formicidae). J Econ Entomol. 94(6):1526-31.

§ Moreno OM (1974). Report to RIFM, 23 August (cited in Opdyke, 1974).

§ Naples JM et al. (1992). Schistosoma mansoni: Cercaricidal effects of cedarwood oil and various of its components. J Trop Med Hyg 95(6), 390-396.

- Noiles K and Pratt M (2010). Contact dermatitis to Vicks VapoRub. Dermatitis 21, 167.
- NTP (2002). US National Toxicology Program. Summary of data for chemical selection. Cedarwood Oil. Available at http://ntp.niehs.nih.gov/ntp/htdocs/chem_background/exsumpdf/cedarwood_oil_508.pdf
- NTP (2005a). US National Toxicology Program. 90-day topical application study (study ID C20316). Avaiable at http://ntp.niehs.nih.gov/testing/status/agents/ts-m030012.html
- NTP (2005b). US National Toxicology Program. Micronucleus assay (study ID A16009). Available at http://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-01843-0001-0000-9
- NTP (2007). US National Toxicology Program. Salmonella assay (study ID A51043). Available at http://tools.niehs.nih.gov/cebs3/ntpViews/?studyNumber=002-01843-0002-0000-0
- OECD (undated). Organisation for Economic Cooperation and Development. The Global Portal to Information on Chemical Substances (eChemPortal). Oils, cedarwood, Texan; Oils, cedarwood. Accessed March 2017. Available at: http://webnet.oecd.org/CCRWeb/Search.aspx
- Ogata A et al. (1999) Teratogenicity of thujaplicin in ICR mice. Food & Chem. Toxicol., 37, 1097-1104 (cited in NTP, 2002).
- Opdyke, DLJ (ed.) (1974). Monographs on Fragrance Raw Materials: Cedarwood oil Virginia. Fd Cosmetics Toxicol. 12, 845-6.
- Opdyke, DLJ (ed.) (1976). Monographs on Fragrance Raw Materials: Cedarwood oil Texas. Fd Cosmetics Toxicol. 14, 711-2.
- Renne RA *et al.* (2006). Effects of flavoring and casing ingredients on the toxicity of mainstream cigarette smoke in rats. Inhal. Toxicol. 18, 685-706.
- Roe FJC and Field WEH (1965). Chronic toxicity of essential oils and certain other products of natural origin. Fd Cosmet. Toxicol. 3, 311.
- 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.
- RTECS (2003). Registry of Toxic Effects of Chemical Substances. Record for cedarwood oil (CAS RN 8000-27-9). Last updated August 2003. Accessed March 2017.
- Sabine J R et al. (1973). Spontaneous tumors in C3H-Avy and C3H-AvyfB mice: high incidence in the United States and low incidence in Australia. Journal of the National Cancer Institute, 50, 1237-1242.
- Sabine J R (1975). Exposure to an environment containing the aromatic red cedar, Juniperus virginiana: procarcinogenic, enzyme-inducing and insecticidal effects. Toxicology, 5, 221-235.
- Salaman MH (1961). Acta Un. int. Cancer 17, 12.
- Sax NI (1979). Dangerous properties of industrial materials. 5th Edition. NY: Van Nostrand Reinhold, 455.
- 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.

§ Shorey HH et al. (1993). Disruption of Foraging by Formica aerata (Hymenoptera: Formicidae) Through the Use of Semiochemicals and Related Chemicals. Environ. Entomol. 22(5): 920-924 (cited in US EPA, 2012).

§ Temesvari E *et al*. (2002). Fragrance sensitization caused by temporary henna tattoo. Contact Dermatitis, 47, 240.

§ Urbach F & Forbes PD (1973). Report to RIFM, 18 July (cited in Opdyke, 1974).

§ US Department of Health and Human Services (2016). Household Products Database. Last updated September 2016. Accessed March 2017. Available at http://hpd.nlm.nih.gov/index.htm

§ US EPA (2010). US Environmental Protection Agency. Preliminary product chemistry and human health assessment for the registration review of wood oil. 28 January 2010. Available at http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2009-0258-0008

§ US EPA 2012 CDR list (Chemical Data Reporting Rule). Accessed March 2017. Available at http://iaspub.epa.gov/sor_internet/registry/substreg/searchandretrieve/searchbylist/search.do

§ US EPA Inert Finder Database (2017). Last updated 22 February 2017. Accessed March 2017. Available at https://iaspub.epa.gov/apex/pesticides/f?p=101:1

§ US EPA TSCA inventory. Accessed March 2017. Available at http://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.

§ Vlahakis G (1977). Possible carcinogenic effects of cedar shavings in bedding of C3H-Avy fB mice. J. Natl Cancer Inst. 58(1): 149-50.

§ Wade AE *et al.* (1968). Alteration of drug metablolism in rats and mice by an environment of cedarwood. Pharmacology, 1, 317-328 (cited in NTP, 2002).

§ Yang MH et al. (2013). Screening of medicinal plants for PPARα and PPARγ activation and evaluation of their effects on glucose uptake and 3T3-L1 adipogenesis. Planta Med. 79(12), 1084-95. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23877921

§ Yeung & Foster (2003). Encyclopaedia of common ingredients used in food, drugs and cosmetics, 2nd edition.

11. Other Information

12. Last Audited

May 2017

United States
Environmental Protection
Agency

Prevention, Pesticides,
And Toxic Substances
(H7508W)

EPA 738-S-93-012
September 1993

EPA

Reregistration Eligibility Decision (RED)

Cedarwood Oil

TABLE OF CONTENTS

Appendix E - Pesticide Reregistration Handbook

Appendix F - Generic Data Call-In

Attachment A -	Chemical Status Sheet
Attachment B -	Generic DCI Response Forms (Form A) plus Instructions
Attachment C -	Requirements Status and Registrants' Response Forms (Form B) plus Instructions
Attachment D -	List of all Registrant(s) sent this DCI

Appendix G - Product Specific Data Call-In

Attachment A -	Chemical Status Sheet
Attachment B -	Product Specific DCI Response Forms (Form A) plus Instructions
Attachment C -	Requirements Status and Registrants' Response Forms (Form B) plus Instructions
Attachment D -	EPA Grouping of End Use Products for meeting Acute Toxicology Data Requirements.
Attachment E -	EPA Acceptance Criteria
Attachment F -	List of all Registrant(s) sent this DCI
Attachment G -	Cost Share/Data Compensation Forms

WOOD OILS AND GUMS REREGISTRATION ELIGIBILITY DECISION TEAM

Office of Pesticide Programs:

Biological and Economic Analysis Division

Janet Andersen	Biological Analysis Branch

Environmental Fate and Effects Division

William Schneider	Science Analysis and Coordination Staff

Health Effects Division

Flora Chow	Chemical Coordination Branch
Tom McClintock	Science Analysis Branch
Charles Frick	Chemical Coordination Branch

Registration Division

Marion Johnson, Jr.	Insecticide and Rodenticide Branch
Al Smith	Registration Support Branch
JoAnne Hayes	Registration Support Branch

Special Review and Reregistration Division

Virginia Dietrich	Accelerated Reregistration Branch
Bruce Sidwell	Accelerated Reregistration Branch

Office of Compliance Monitoring:

Phyllis Flaherty	Pesticides Enforcement Policy Branch

Office of General Counsel:

Kevin Lee	Pesticides Branch

GLOSSARY OF TERMS AND ABBREVIATIONS

a.i.	Active Ingredient
CAS	Chemical Abstracts Service
CSF	Confidential Statement of Formula
EEC	Estimated Environmental Concentration. The estimated pesticide concentration in an environment, such as a terrestrial ecosystem.
EP	End-Use Product
EPA	U.S. Environmental Protection Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FFDCA	Federal Food, Drug, and Cosmetic Act
HDT	Highest Dose Tested
LC_{50}	Median Lethal Concentration. A statistically derived concentration of a substance that can be expected to cause death in 50% of test animals. It is usually expressed as the weight of substance per weight or volume of water or feed, e.g., mg/l or ppm.
LD_{50}	Median Lethal Dose. A statistically derived single dose that can be expected to cause death in 50% of the test animals when administered by the route indicated (oral, dermal). It is expressed as a weight of substance per unit weight of animal, e.g., mg/kg.
LD_{lo}	Lethal Dose-low. Lowest Dose at which lethality occurs
LEL	Lowest Effect Level
LOEL	Lowest Observed Effect Level
MP	Manufacturing-Use Product
MPI	Maximum Permissible Intake
MRID	Master Record Identification (number). EPA's system of recording and tracking studies submitted.

GLOSSARY OF TERMS AND ABBREVIATIONS (cont.)

N/A	Not Applicable
NPDES	National Pollutant Discharge Elimination System
NOEL	No Observed Effect Level
OPP	Office of Pesticide Programs
PADI	Provisional Acceptable Daily Intake
ppm	Parts Per Million
RfD	Reference Dose
RS	Registration Standard
TD	Toxic Dose. The dose at which a substance produces a toxic effect.
TC	Toxic Concentration. The dose at which a substance produces a toxic effect.
TMRC	Theoretical Maximum Residue Contribution.

EXECUTIVE SUMMARY

The Agency has completed its reregistration assessment of the available information on the pesticide active ingredient cedarwood oil in the case named Wood Oils and Gums. It has been determined that the currently registered uses will not cause unreasonable risk to humans or the environment and these uses are eligible for reregistration.

Cedarwood oil is a natural component of wood from the tree, Juniperus virginiana L., for use in pesticide products to repel moths from and inhibit mildew in clothing; and repel fleas from pets and their sleeping quarters. Current registered products include cedarwood blocks, a pet collar, and a ready-to-use liquid. The Agency is proposing, under a separate action, to deregulate the cedarwood block products in accordance with FIFRA 25(b).

Before reregistering the products containing cedarwood oil, the Agency is requiring that additional technical chemistry data on the extracted oil, as well as product specific data on acute toxicology, chemistry and efficacy, revised Confidential Statements of Formula (CSF) and revised labeling be submitted within eight months of the issuance of this document. After reviewing these data and revised labels and finding them acceptable in accordance with Section 3(c)(5) of FIFRA, the Agency will reregister products. Those products which contain other active ingredients will be eligible for reregistration only when the other active ingredients are determined to be eligible for reregistration.

I. INTRODUCTION

In 1988, the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) was amended to accelerate the reregistration of products with active ingredients registered prior to November 1, 1984. The amended Act provides a schedule for the reregistration process to be completed in nine years. There are five phases to the reregistration process. The first four phases of the process focus on identification of data requirements to support the reregistration of an active ingredient and the generation and submission of data to fulfill the requirements. The fifth phase is a review by the U.S. Environmental Protection Agency (referred to as "the Agency") of all data submitted to support reregistration.

FIFRA Section 4(g)(2)(A) states that in Phase 5 "the Administrator shall determine whether pesticides containing such active ingredient are eligible for reregistration" before calling in data on products and either reregistering products or taking other "appropriate regulatory action." Thus, reregistration involves a thorough review of the scientific data base underlying a pesticide's registration. The purpose of the Agency's review is to reassess the potential hazards arising from the currently registered uses of the pesticide; to determine the need for additional data on health and environmental effects; and to determine whether the pesticide meets the "no unreasonable adverse effects" criterion of FIFRA.

This document presents the Agency's decision regarding the reregistration eligibility of the registered uses of cedarwood oil. The document consists of six sections. Section I is the introduction. Section II describes cedarwood oil, its uses, data requirements and regulatory history. Section III discusses the human health and environmental assessment based on the data available to the Agency. Section IV presents the reregistration decision for cedarwood oil. Section V discusses the reregistration requirements for cedarwood oil . Finally, Section VI is the Appendices which support this Reregistration Eligibility Decision document. Additional details concerning the Agency's review of applicable data are available on request.[1]

[1]EPA's reviews of data on the set of registered uses considered for EPA's analysis may be obtained from the OPP Public Docket, Field Operations Division (H7506C), Office of Pesticide Programs, EPA, Washington, DC 20460.

II. CASE OVERVIEW

A. Chemical Overview

The following active ingredient is covered by this Reregistration Eligibility Decision document:

- **Common Name:** Cedarwood oil
- **Chemical Name:** Oil extracted from species of cedar trees, especially Juniperus virginiana L. (1)
- **Chemical Family:** Wood oils and gums
- **CAS Registry Number:** 800-27-9
- **OPP Chemical Code:** 40505
- **Empirical Formula:** cedarwood oil is a mixture of organic compounds
- **Trade and Other Names:** oil of cedar
- **Basic Manufacturer:** not applicable

B. Use Profile

The following is information on the current registered uses with an overview of use sites and application methods. Appendix A is a detailed table of these uses of cedarwood oil.

For Cedarwood oil:

Type of Pesticide: repellant/feeding depressant; fungicide

Use Sites: Indoor Residential - domestic dwellings and their contents, Pets/Animals (presumably cats and dogs) and their living and sleeping quarters

Target Pests: fleas, moths, mildew

Formulation Types Registered:

Liquid ready-to-use - sprayed on animal bedding

0.48 % cedarwood oil

Impregnated pet collar/tag

0.5 % oil of citronella
1.0 % oil of eucalyptus
0.5 % cedarwood oil
2.0 % oil of pennyroyal
0.125 % oils, rue;

Wood blocks, containing an average of --

5.17% cedarwood oil (2-8% range)

C. Data Requirements

The Agency has waived all generic data requirements except for physical chemistry for this active ingredient. The rationale for this action is discussed below and also in Section III. Instead it has relied on general, commonly available information about cedarwood oil. Appendix B includes all data requirements identified by the Agency for currently registered uses needed to support reregistration.

EPA has developed a target database, set forth in the regulations (2) and the Agency's Reregistration Phase 2 Technical Guidance Document, to be addressed for pesticide reregistration. These regulations and the Guidance Document specify the necessary data based on factors including use sites, potential environmental and human (dietary and occupational) exposures, product formulation types, and product application methods. Due to the diverse nature and characteristics of pesticide products and their uses subject to reregistration, the Agency also recognizes the necessity to modify the data requirements for specific pesticides, including waiving certain data requirements because such requirements are inappropriate or unnecessary for risk assessment and reregistration.

This approach to waiving individual data requirements has served to identify the appropriate data requirement sets for pesticide products. Further, the Agency believes there is a category of pesticide active ingredients for which a broadly reduced set of data requirements are appropriate for reregistration. Specifically, products in this category may be exempt from the generic data requirements for toxicology, residue chemistry, human exposure, ecological effects, and environmental fate on the active ingredient. The Agency believes there are considerations which, when taken together, can form the basis for a conclusion that such a reduction in data requirements is appropriate for a particular pesticide active ingredient, while not compromising human health or environmental safety.

There are, however, certain data requirements which are essential and not likely to be waived. Basic product identity/chemistry information on the active ingredient and formulated products is required for pesticides in this category so that the Agency has reasonable certainty of the pesticide's chemical and physical characteristics. Also, product specific acute toxicology studies are required for the Agency to determine appropriate product labeling for potential hazards to those who handle or apply these products. However, these toxicology studies may also be waived if an assessment of the product formulation, including the inert ingredients, indicates that such studies are unnecessary to prescribe appropriate labeling. Efficacy studies may be required of formulated products labeled for uses and pests that are of a public health concern.

In considering cedarwood oil for reregistration eligibility the Agency believes it is an active ingredient that should be considered for this broad waiver of the generic data requirements. The considerations that led the Agency to this conclusion are discussed in Section III below.

D. Regulatory History

Originally, the reregistration case Wood Oil and Gums included three active ingredients: cedarwood oil, canadian balsam, and ester gum. After Phase 2 in 1990, products containing the latter two active ingredients were cancelled for non-support by the registrants, leaving only products containing cedarwood oil.

Cedarwood oil was initially registered as a pesticide in the United States in 1960 to repel moths from clothing. Since then products have been registered to repel fleas from pets and their bedding. The five currently registered products containing cedarwood oil as an active ingredient fall under one of three types of pesticide products: three are solid cedarwood block products, ("Cedar Fresh", EPA Reg. No. 65555-1; "Ozark Cedar", EPA Reg. No. 65813-1, and "Woodland/RPM Cedar", EPA Reg. No. 66211-1), one product is a pet collar ("Herbal Flea Collar", EPA Reg. No. 42443-1), and one product is a ready-to-use liquid that may be applied by hand spray ("Green Earth

Flea Spray" Reg. No. 63380-1).

III. SCIENCE ASSESSMENT

A. Physical Chemistry Assessment

Cedarwood oil is a distilled extract from the cured cedarwood obtained from Juniperus virginiana L., and other species of cedar. The chief chemical components are cedrene (a terpene) and cedral (cedar camphor).

Cedarwood oil ranges in color from colorless to slightly yellow. It is somewhat viscid. It is insoluble in water but soluble in both alcohol and ether (1).

B. Human Health And Environmental Assessment

As discussed above, the Agency has waived the generic data requirements, except for certain technical chemistry information, for cedarwood oil. It is relying on commonly available information about this chemical and its uses to reach a decision about its potential risks to human health and the environment associated with the current uses of registered products. The specific information about cedarwood oil the Agency considered is as follows:

Cedarwood oil is a mixture of organic compounds. It is a component of many non-pesticidal consumer products currently marketed in the United States. Cedarwood oil is listed as a food additive by the Food and Drug Administration (5). The alcohols and terpenes of cedarwood oil are considered by FDA to be Generally Recognized As Safe (GRAS). As a pesticide, it repels insect pests by a non-toxic mode of action. Its mode of action against mildew is unknown. EPA is not aware of any adverse effects of the active ingredient to humans or the environment in the literature when used in a manner prescribed in end-use product labeling. There have been no reported incidents of toxicity.

There have been some studies performed on cedarwood dust as well as the constituents of cedarwood oil. According to a recent survey of the literature, pulmonary effects and liver effects have been noted in laboratory animals (3). These effects are hypothesized to be related to the occupational hazards associated with saw mill workers (4) chronically exposed to environments high in cedarwood dust. However, this type of exposure is not indicative of that from use of the currently registered pesticide products.

The Agency believes there is negligible human and environmental exposure to the pesticide as a result of the use patterns; there is a low use rate and frequency of

application and/or the products are applied in a confined or contained manner. However, the handling and use of the liquid product could pose a relatively greater exposure potential by the dermal and inhalation routes. Product specific acute toxicity testing will allow the Agency to address appropriate labeling to address potential concerns for users.

Since the pesticide will be used in indoor domestic dwellings, on pets and on their living and sleeping quarters, the Agency expects that there will be negligible exposure to the environment and to nontarget organisms.

Based on these factors the Agency does not believe generic data, beyond those data required to satisfy basic characterization of the chemistry (refer to Appendix B), are necessary to determine whether the current registered uses of this active ingredient pose unreasonable risks to humans or the environment. However, it is requiring the submission of product specific data (chemistry, acute toxicity and efficacy).

In conclusion, the Agency has determined that the use of cedarwood oil as an active ingredient in products for the current uses should not result in unreasonable adverse effects to human health or the environment.

IV. RISK MANAGEMENT AND REREGISTRATION ELIGIBILITY DECISION

A. Determination of Reregistration Eligibility

Section 4(g)(2)(A) of FIFRA calls for the Agency to determine, after submission of relevant data concerning an active ingredient, whether products containing the active ingredients are eligible for reregistration. As discussed above, the Agency has determined that the set of generic data requirements that would normally be applicable to cedarwood oil need not be satisfied for the Agency to reach a decision on potential risks and reregistration eligibility. Rather, it has considered general and commonly available information. The Agency has determined that cedarwood oil meets criteria as outlined in the document "Guidance for Making Determinations to Reduce Data Requirements". Cedarwood oil met the criteria due to its use and availability for non-pesticide food uses, its regulatory status as a chemical classified as GRAS and exempt from the requirement of food additive tolerances; its non-toxic mode of action as a pesticide; that there is negligible human and environmental exposure to the pesticide as a result of the porposed use pattern, and the lack of reports of adverse effects. (No data were submitted under 6(a)(2) of FIFRA, no significant incidents have been reported to the Agency, and there is no indication in the literature that the pesticide poses adverse effects in humans or to the environment when used in a manner prescribed in end-use poduct labeling.) Appendix B identifies the sources for this information that the Agency reviewed as part of its determination of reregistration eligibility of cedarwood oil and

lists the submitted studies that the Agency considered acceptable.

The Agency believes this information is sufficient to support reregistration and, that cedarwood oil can be used without resulting in unreasonable adverse effects to human health and the environment. The Agency therefore finds that all products containing cedarwood oil as the active ingredient are eligible for reregistration. The reregistration of particular products is addressed in Section V of this document.

Although the Agency has found that all uses of cedarwood oil are eligible for reregistration, it should be understood that the Agency may take appropriate regulatory action, and/or require the submission of additional data to support the registration of products containing cedarwood oil, if new information comes to the Agency's attention or if the data requirements for registration (or the guidelines for generating such data) change.

However, the Agency has determined that natural cedarwood products consisting of wood blocks, not treated or impregnated with any additional substance(s), distributed and sold as moth and flea repellents or mildew control agents, are of a character which is unnecessary to be subject to FIFRA. Cedarwood oil is naturally contained in the cedar wood; it cannot easily be separated from the wood or wood products. Consumers using these cedarwood products are unlikely to be exposed to significant amounts of cedarwood dust or oil, either by the inhalation or dermal route. The Agency believes there is negligible risk to man or the environment associated with the use of these wood products as pesticides. Therefore, under authority of FIFRA section 25(b), 7 U.S.C 136w(b), the Agency has proposed that such products be exempted from provisions of FIFRA, except misbranding provisions (58 FR 42711; August 11, 1993). A 30-day comment period is provided. Products containing cedarwood oil extracted from wood or synthesized and subsequently used for pesticide purposes are not included in this proposed exemption.

Since the Agency is proposing that these cedarwood products be exempted from regulation, further reregistration requirements are being held in abeyance. If the Agency concludes it should not proceed to effect the exemption of these products, the Agency will proceed to impose the appropriate reregistration requirements on these products.

1. Eligible and Ineligible Uses

The Agency has determined that all current uses of cedarwood oil products are eligible for reregistration.

B. Risk Management Decision

In consideration of the above information about cedarwood oil, the Agency finds no reason to impose new risk reduction measures for currently registered uses. The

Agency will however, assess the need for product specific risk reduction measures upon receipt of data that are being required under the Product Specific Data Call-in Notice appended to this document.

V. ACTIONS REQUIRED BY REGISTRANTS

This section specifies the data requirements and responses necessary for the reregistration of end-use products. Because there are no registered manufacturing-use products or technical products the generic data requirements are required for all registrants.

A. Technical Grade Information

1. Generic Data Requirements

There are currently no registered manufacturing use products for cedarwood oil. The generic data base supporting the reregistration of products containing cedarwood oil for the above eligible uses has been reviewed and determined to be incomplete. Registrants are required to submit the technical chemistry data corresponding to Series 61 and Series 62 for the analysis and certification of product ingredients. If the product is a United States Pharmacopoeia (USP) grade, a copy of USP analysis with citation of the analytical methods used and certification would satisfy the requirement for Series 62.

The Confidential Statement of Formula (CSF) must be supported by analytical data. The data on the physical and chemical characteristics of cedarwood oil from the Material Safety Data Sheet (MSDS) for the product may be compiled by the registrant in the format required by the FIFRA Accelerated Reregistration Phase 3 Technical Guidedance, specifically PR Notice 86-5 to satisfy some of the requirements of Series 63. The generic data requirements are listed in Appendix F, the Generic Data Call-in Notice.

B. End-Use Products

1. Additional Product-Specific Data Requirements

Section 4(g)(2)B) of FIFRA calls for the Agency to obtain any needed product-specific data regarding the pesticide after a determination of eligibility has been made. The product specific data requirements are listed in Appendix G, the Product Specific Data Call-In Notice.

Registrants must review previous data submissions to ensure that they meet current EPA acceptance criteria (Appendix F; Attachment E) and if not, commit to conduct new studies. If a registrant believes that previously submitted data meet current testing standards, then study MRID numbers should be cited according to the instructions in the Requirement Status and Registrants Response Form provided for each product.

Additional physical chemistry data on cedarwood oil are required to provide the Agency with a more complete characterization of the chemistry of the cedarwood oil that is used in the registered products. These generic data requirements are imposed on the end-use products because there is no registered source of technical grade cedarwood oil.

2. Labeling Requirements for End-Use Products

The labels and labeling of all products must comply with EPA's current regulations and requirements as specified in 40 CFR §156.10. Please follow the instructions in the Pesticide Reregistration Handbook with respect to labels and labeling. In addition, registrants with products that are used on pets and/or animals will be required to specify on their labels which animals may be treated with the product.

A. Existing Stocks

Registrants may generally distribute and sell products bearing old labels/labeling for 26 months from the date of the issuance of this Reregistration Eligibility Decision document (RED). Persons other than the registrant may generally distribute or sell such products for 50 months from the date of the issuance of the RED. However, existing stocks time frames will be established case-by-case, depending on the number of products involved, the number of label changes, and other factors. Refer to "Existing Stocks of Pesticide Products; Statement of Policy"; Federal Register, Volume 56, No. 123, June 26, 1991.

The Agency has determined that registrants may distribute and sell cedarwood oil products bearing old labels/labeling for 26 months from the date of issuance of this RED. Persons other than the registrant may distribute or sell such products for 50 months from the date of the issuance of this RED.

VI.
APPENDICES

APPENDIX A

Table of Use Patterns Subject to Reregistration

SITE Application Type, Application Timing, Application Equipment - Surface Type & Efficacy Influencing Factor (Antimicrobial only)	Form	Minimum Application Rate	Maximum Application Rates	Soil Text (Max Dse)	Max # Appa	Max. Appa @ Max Rate	Min. Interv (days)	Restr. Entry Interv (days)	Geographic Allowed	Geographic Disallowed	Use Limitations Codes
USES ELIGIBLE FOR REREGISTRATION											
NON-FOOD/NON-FEED											
HOUSEHOLD/DOMESTIC DWELLINGS CONTENTS			Use Group: INDOOR RESIDENTIAL								
Fumigation., When needed., By hand.	IMPR	NA	NA	*	NS	NS	NS	NS			
HOUSEHOLD/DOMESTIC DWELLINGS INDOOR PREMISES			Use Group: INDOOR RESIDENTIAL								
Fumigation., When needed., By hand.	IMPR	NA	NA	*	NS	NS	NS	NS			
PET LIVING/SLEEPING QUARTERS			Use Group: INDOOR RESIDENTIAL								
Animal bedding treatment., When needed., Pump spray bottle.	RTU	NA	NA	*	NS	NS	AN	NS			
Fumigation., When needed., By hand.	IMPR	NA	NA	*	NS	NS	NS	NS			
SITE TERM TOO GENERAL			Use Group: INDOOR RESIDENTIAL								
Animal treatment (spray)., When needed., Pump spray bottle.	RTU	NA	NA	*	NS	NS	AN	NS			
Flea collar., When needed., By hand.	IC/T	NA	.0003125 lb animal	*	NS	NS	NS	NS			

LEGEND

HEADER ABBREVIATIONS
Max. # Apps : Maximum number of Applications
Max. Apps @ Max Rate : Maximum number of Applications at Maximum Dosage Rate
Min. Interv (days) : Minimum Interval between Applications (days)
Restr. Entry Interv (days) : Restricted Entry Interval (days)

SOIL TEXTURE FOR MAX APP. RATE
* : Non-specific
C : Coarse
M : Medium
F : Fine
O : Others

FORMULATION CODES
IC/T : IMPREGNATED COLLAR/TAG
IMPR : IMPREGNATED MATERIAL
RTU : LIQUID-READY TO USE

ABBREVIATIONS
AN : As Needed
NA : Not Applicable
NS : Not Specified (on label)

APPLICATION RATE
DCNC : Dosage Can Not be Calculated
No Calc : No Calculation can be made
W : PPM calculated by weight
V : PPM Calculated by volume
cwt : Hundred Weight

APPENDIX B

Table of the Generic Data Requirements and Studies Used to Make the Reregistration Decision

GUIDE TO APPENDIX B

Appendix B contains listings of data requirements which support the reregistration for the pesticide bromine covered by this Reregistration Eligibility Decision. It contains generic data requirements that apply to bromine in all products, including data requirements for which a "typical formulation" is the test substance.

The data table is organized in the following format:

1. Data Requirement (Column 1). The data requirements are listed in the order in which they appear in 40 CFR, Part 158. The reference numbers accompanying each test refer to the test protocols set in the Pesticide Assessment Guidelines, which are available from the National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161 (703) 487 - 4650.

2. Use Pattern (Column 2). This column indicates the use patterns for which the data requirements apply. The following letter designations are used for the given use patterns:

A	Terrestrial food
B	Terrestrial feed
C	Terrestrial non-food
D	Aquatic food
E	Aquatic non-food outdoor
F	Aquatic non-food industrial
G	Aquatic non-food residential
H	Greenhouse food
I	Greenhouse non-food
J	Forestry
K	Residential
L	Indoor food
M	Indoor non-food
N	Indoor medical
O	Indoor residential

3. Bibliographic citation (Column 3). If the Agency has acceptable data in its files, this column lists the identifying number of each study. This normally is the Master Record Identification (MRID) number, but may be a "GS" number if no MRID number has been assigned. Refer to the Bibliography appendix for a complete citation of the study.

APPENDIX B

Data Supporting Guideline Requirements for the Reregistration of Cedarwood Oil

REQUIREMENT		USE PATTERN	CITATION
PRODUCT CHEMISTRY			
61-1	Chemical Identity	All	42319001, 42101201, 41549503
61-2A	Start. Mat. & Mnfg. Process	All	42101201, 42362601, 42319001
61-2B	Formation of Impurities	All	42101201, 42362601, 42319001
62-1	Preliminary Analysis	All	42362602, 42319002
62-2	Certification of limits	All	42362602, 42319002
62-3	Analytical Method	All	42319002
63-2	Color	All	SATISFIED
63-3	Physical State	All	SATISFIED
63-4	Odor	All	SATISFIED
63-5	Melting Point	All	SATISFIED
63-6	Boiling Point	All	SATISFIED
63-7	Density	All	SATISFIED
63-8	Solubility	All	SATISFIED
63-9	Vapor Pressure	All	SATISFIED
63-10	Dissociation Constant	ALL	SATISFIED
63-11	Octanol/Water Partition	ALL	SATISFIED
63-12	pH	ALL	SATISFIED

APPENDIX B

Data Supporting Guideline Requirements for the Reregistration of Cedarwood Oil

REQUIREMENT		USE PATTERN	CITATION
PRODUCT CHEMISTRY			
63-13[1]	Stability	ALL	SATISFIED
ECOLOGICAL EFFECTS			
71-1A	Acute Avian Oral - Quail/Duck	ALL	WAIVED
71-2A	Avian Dietary - Quail	ALL	WAIVED
72-1A	Fish Toxicity Bluegill	ALL	WAIVED
72-1C	Fish Toxicity Rainbow Trout	ALL	WAIVED
72-2A	Invertebrate Toxicity	ALL	WAIVED
TOXICOLOGY			
81-1	Acute Oral Toxicity - Rat	ALL	WAIVED
81-2	Acute Dermal Toxicity - Rabbit/Rat	ALL	WAIVED
81-3	Acute Inhalation Toxicity - Rat	ALL	WAIVED
81-4	Primary Eye Irritation - Rabbit	ALL	WAIVED
81-5	Primary Dermal Irritation - Rabbit	ALL	WAIVED
81-6	Dermal Sensitization - Guinea Pig	ALL	WAIVED

[1] Confirmatory data are being required from certain registrants

APPENDIX B

Data Supporting Guideline Requirements for the Reregistration of Cedarwood Oil

REQUIREMENT		USE PATTERN	CITATION
TOXICOLOGY			
84-2A	Gene Muatation-(Ames Test)	All	WAIVED
84-2B	Structural Chromosomal Aberration	ALL	WAIVED
84-4	Other Genotoxic Effects	ALL	WAIVED

APPENDIX B

Data Supporting Guideline Requirements for the Reregistration of Cedarwood Oil

REQUIREMENT		USE PATTERN	CITATION
ENVIRONMENTAL FATE			
161-1	Hydrolysis	ALL	WAIVED

APPENDIX C

Citations Considered to be Part of the Data Base Supporting the Reregistration of Wood Oils and Gums

GUIDE TO APPENDIX C

1. CONTENTS OF BIBLIOGRAPHY. This bibliography contains citations of all studies considered relevant by EPA in arriving at the positions and conclusions stated elsewhere in the Reregistration Eligibility Decision. Primary sources for studies in this bibliography have been the body of data submitted to EPA and its predecessor agencies in support of past regulatory decisions. Selections from other sources including the published literature, in those instances where they have been considered, are included.

2. UNITS OF ENTRY. The unit of entry in this bibliography is called a "study". In the case of published materials, this corresponds closely to an article. In the case of unpublished materials submitted to the Agency, the Agency has sought to identify documents at a level parallel to the published article from within the typically larger volumes in which they were submitted. The resulting "studies" generally have a distinct title (or at least a single subject), can stand alone for purposes of review and can be described with a conventional bibliographic citation. The Agency has also attempted to unite basic documents and commentaries upon them, treating them as a single study.

3. IDENTIFICATION OF ENTRIES. The entries in this bibliography are sorted numerically by Master Record Identifier, or "MRID number". This number is unique to the citation, and should be used whenever a specific reference is required. It is not related to the six-digit "Accession Number" which has been used to identify volumes of submitted studies (see paragraph 4(d)(4) below for further explanation). In a few cases, entries added to the bibliography late in the review may be preceded by a nine character temporary identifier. These entries are listed after all MRID entries. This temporary identifying number is also to be used whenever specific reference is needed.

4. FORM OF ENTRY. In addition to the Master Record Identifier (MRID), each entry consists of a citation containing standard elements followed, in the case of material submitted to EPA, by a description of the earliest known submission. Bibliographic conventions used reflect the standard of the American National Standards Institute (ANSI), expanded to provide for certain special needs.

   a Author. Whenever the author could confidently be identified, the Agency has chosen to show a personal author. When no individual was identified, the Agency has shown an identifiable laboratory or testing facility as the author. When no author or laboratory could be identified, the Agency has shown the first submitter as the author.

   b. Document date. The date of the study is taken directly from the document. When the date is followed by a question mark, the bibliographer has deduced the date from the evidence contained in the document. When the date appears as (19??), the Agency was unable to determine or estimate the date of the document.

c. Title. In some cases, it has been necessary for the Agency bibliographers to create or enhance a document title. Any such editorial insertions are contained between square brackets.

d. Trailing parentheses. For studies submitted to the Agency in the past, the trailing parentheses include (in addition to any self-explanatory text) the following elements describing the earliest known submission:

(1) Submission date. The date of the earliest known submission appears immediately following the word "received."

(2) Administrative number. The next element immediately following the word "under" is the registration number, experimental use permit number, petition number, or other administrative number associated with the earliest known submission.

(3) Submitter. The third element is the submitter. When authorship is defaulted to the submitter, this element is omitted.

(4) Volume Identification (Accession Numbers). The final element in the trailing parentheses identifies the EPA accession number of the volume in which the original submission of the study appears. The six-digit accession number follows the symbol "CDL," which stands for "Company Data Library." This accession number is in turn followed by an alphabetic suffix which shows the relative position of the study within the volume.

-------- 1. The Merck Index; An Encyclopedia of Chemicals, Drugs, and Biologicals. Windholz, Martha, editor, et. al. Tenth Edition. Published in 1983 by Merck and Company, Rahway New Jersey, U.S.A.

-------- 2. Code of Federal Regulations, Title 40, part 158, revised as of July 1, 1992. Published by the Office of the Federal Register National Archives and Records Administration, Washington, D.C., U.S.A.

-------- 3. Gordon WP; Forte AJ; McMurtry RJ; Nelson SD (1982) Hepatotoxicity and Pulmonary Toxicity of Pennyroyal Oil and its Constituent Terpenes in the Mouse. Published by the Journal of Toxicology and Applied Pharmacology; Volume 65, ISS 4, P413-24.}

-------- 4. Ayars GH; Altman LC; Frazier CE; Chi EY; (1989) The Toxicity of Constituents of Cedar and Pine Woods to Pulmonary Epithelium. Published by Journal of Allergy and Clinical Immunology; Volume 83, ISS 3, P610.

-------- 5. Code of Federal Regulations, Title 21, part 172, section 515, revised as of July 1, 1992. Published by the Office of the Federal Register National Archives and Records Administration, Washington, D.C., U.S.A.

41549503 Gunther (19??) Oil of Cedarwood. pp. 356-363 in unknown source.

42101201 Arbor American Corp. (1991) Chemical Identity: Cedar Wood Oil. Unpublished study. 6 p.

42319001 Anon. (1992) Product Identification and Disclosure of Ingredients; Description of Beginning Materials and Manufacturing process; Discussion of formation of impurities (contains published material). Unpublished study prepared by Cor-Pak International. 15 p.

42319002 Maltese, L. (1992) Analysis of Redwood Cedars: Lab Project Number: 92-1076: 92-1117: 92-1216. Unpublished study prepared by Stillwell & Gladding. 13 p.

42362601 Anon. (1992) Description of Materials Used to Produce Product and Description of Production Process: Aromatic Cedar. Unpublished study prepared by P & M Consumer Products, Inc. 7 p.

42362602 Zavarin, E. (1992) Analysis of Red Cedar Wood Juniperus Virginiana: Unpublished study prepared by Forest Products Laboratory (University of California at Berkley). 8 p.

APPENDIX D
List of Available Related Documents

The following is a list of available documents related to cedarwood oil. It's purpose is to provide a path to more detailed information if it is needed. These accompanying documents are part of the Administrative Record for cedarwood oil and are included in the EPA's Office of Pesticide Programs Public Docket.

1. Health and Environmental Effects Science Chapters

2. Detailed Label Usage Information System (LUIS) Report

3. Cedarwood oil RED Fact Sheet

4. PR Notice 91-2 (included in this appendix) pertains to the Label Ingredient Statement

5. Guidance for Making Determinations to Reduce Data Requirements

6. Minutes from the April 19 and May 17 meetings of the Ad Hoc Screening Committee for Reduced Data Requirements

APPENDIX E

Pesticide Reregistration Handbook
PR Notices 86-5 and 91-2

PESTICIDE REGISTRATION HANDBOOK

PR Notice 86-5

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

July 29, 1986

PR NOTICE 86-5

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

NOTICE TO PRODUCERS, FORMULATORS, DISTRIBUTORS AND REGISTRANTS

Attention: Persons responsible for Federal registration of pesticides.

Subject: Standard format for data submitted under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and certain provisions of the Federal Food, Drug, and Cosmetic Act (FFDCA).

I. Purpose

To require data to be submitted to the Environmental Protection Agency (EPA) in a standard format. This Notice also provides additional guidance about, and illustrations of, the required formats.

II. Applicability

This PR Notice applies to all data that are submitted to EPA to satisfy data requirements for granting or maintaining pesticide registrations, experimental use permits, tolerances, and related approvals under certain provisions of FIFRA and FFDCA. These data are defined in FIFRA §10(d)(1). This Notice does not apply to commercial, financial, or production information, which are, and must continue to be, submitted differently under separate cover.

III. Effective Date

This notice is effective on November 1, 1986. Data formatted according to this notice may be submitted prior to the effective date. As of the effective date, submitted data packages that do not conform to these requirements may be returned to the submitter for necessary revision.

IV. Background

On September 26, 1984, EPA published proposed regulations in the Federal Register (49 FR 37956) which include Requirements for Data Submission (40 CFR §158.32), and Procedures for Claims of Confidentiality of Data (40 CFR §158.33). These regulations

specify the format for data submitted to EPA under Section 3 of FIFRA and Sections 408 and 409 of FFDCA, and procedures which must be followed to make and substantiate claims of confidentiality. No entitlements to data confidentiality are changed, either by the proposed regulation or by this notice.

OPP is making these requirements mandatory through this Notice to gain resource-saving benefits from their use before the entire proposed regulation becomes final. Adequate lead time is being provided for submitters to comply with the new requirements.

V. Relationship of this Notice to Other OPP Policy and Guidance

While this Notice contains requirements for organizing and formatting submittals of supporting data, it does not address the substance of test reports themselves. "Data reporting" guidance is now under development in OPP, and will specify how the study objectives, protocol, observations, findings, and conclusions are organized and presented within the study report. The data reporting guidance will be compatible with submittal format requirements described in this Notice.

OPP has also promulgated a policy (PR Notice 86-4 dated April 15, 1986) that provides for early screening of certain applications for registration under FIFRA §3. The objective of the screen is to avoid the additional costs and prolonged delays associated with handling significantly incomplete application packages. As of the effective date of this Notice, the screen will include in its criteria for acceptance of application packages the data formatting requirements described herein.

OPP has also established a public docket which imposes deadlines for inserting into the docket documents submitted in connection with Special Reviews and Registration Standards (see 40 CFR §154.15 and §155.32). To meet these deadlines, OPP is requiring an additional copy of any data submitted to the docket. Please refer to Page 10 for more information about this requirement.

For several years, OPP has required that each application for registration or other action include a list of all applicable data requirements and an indication of how each is satisfied--the statement of the method of support for the application. Typically, many requirements are satisfied by reference to data previously submitted--either by the applicant or by another party. That requirement is not altered by this notice, which applies only to data submitted with an application.

VI. Format Requirements

A more detailed discussion of these format requirements follows the index on the next page, and samples of some of the requirements are attached. Except for the language of the two alternative forms of the Statement of Data Confidentiality Claims (shown in Attachment 3) which cannot be altered, these samples are illustrative. As long as the required information is included and clearly identifiable, the form of the samples may be altered to reflect the submitter's preference.

- INDEX-

****

A. Organization of Submittal Package

A "submittal package" consists of all studies submitted at the same time for review in support of a single regulatory action, along with a transmittal document and other related administrative material (e.g. the method of support statement, EPA Forms 8570-1, 8570-4, 8570-20, etc.) as appropriate.

Data submitters must organize each submittal package as described in this Notice. The transmittal and any other administrative material must be grouped together in the first physical volume. Each study included in the submittal package must then be bound separately.

Submitters sometimes provide additional materials that are intended to clarify, emphasize, or otherwise comment to help Product Managers and reviewers better understand the submittal.

- If such materials relate to one study, they should be included as an appendix to that study.

- If such materials relate to more than one study (as for example a summary of all studies in a discipline) or to the submittal in general, they must be included in the submittal package as a separate study (with title page and statement of confidentiality claims).

B. Transmittal Document

The first item in each submittal package must be a transmittal document. This document identifies the submitter or all joint submitters; the regulatory action in support of which the package is being submitted--i.e., a registration application, petition, experimental use permit (EUP), §3(c)(2)(B) data call-in, §6(a)(2) submittal, or a special review; the transmittal date; and a list of all individual studies included in the package in the order of their appearance, showing (usually by Guideline reference number) the data requirement(s) addressed by each one. The EPA-assigned number for the regulatory action (e.g. the registration, EUP, or tolerance petition number) should be included in the transmittal document as well, if it is known to the submitter. See Attachment 1 for an example of an acceptable transmittal document.

The list of included studies in the transmittal of a data submittal package supporting a registration application should be subdivided by discipline, reflecting the order in which data requirements appear in 40 CFR 158.

The list of included studies in the transmittal of a data submittal package supporting a petition for tolerance or an application for an EUP should be subdivided into sections A, B, C,.... of the petition or application, as defined in 40 CFR 180.7 and 158.125, (petitions) or Pesticide Assessment Guidelines, Subdivision I (EUPs) as appropriate.

When a submittal package supports a tolerance petition and an application for a registration or an EUP, list the petition studies first, then the balance of the studies. Within these two groups of studies follow the instructions above.

C. Individual Studies

A study is the report of a single scientific investigation, including all supporting analyses required for logical completeness. A study should be identifiable and distinguishable by a conventional bibliographic citation including author, date, and title. Studies generally correspond in scope to a single Guideline requirement for supporting data, with some exceptions discussed in section C.1. Each study included in a submittal package must be bound as a separate entity. (See comments on binding studies on page 9.)

Each study must be consecutively paginated, beginning from the title page as page 1. The total number of pages in the complete study must be shown on the study title page. In addition (to ensure that inadvertently separated pages can be reassociated with the proper study during handling or review) use either of the following:

- Include the total number of pages in the complete study on each page (i.e., 1 of 250, 2 of 250, ...250 of 250).

- Include a company name or mark and study number on each page of the study, e g , Company Name-1986-23. Never reuse a study number for marking the pages of subsequent studies.

When a single study is extremely long, binding it in multiple volumes is permissible so long as the entire study is paginated in a single series, and each volume is plainly identified by the study title and its position in the multi-volume sequence.

C.1 Special Considerations for Identifying Studies

Some studies raise special problems in study identification, because they address Guidelines of broader than normal scope or for other reasons.

a. Safety Studies. Several Guidelines require testing for safety in more than one species. In these cases each species tested should be reported as a separate study, and bound separately.

Extensive supplemental reports of pathology reviews, feed analyses, historical control data, and the like are often associated with safety studies. Whenever possible these should be submitted with primary reports of the study, and bound with the primary study as appendices. When such supplemental reports are submitted independently of the primary report, take care to fully identify the primary report to which they pertain.

Batteries of acute toxicity tests, performed on the same end use product and covered by a single title page, may be bound together and reported as a single study.

b. Product Chemistry Studies. All product chemistry data within a submittal package submitted in support of an end-use product produced from registered manufacturing-use products should be bound as a single study under a single title page.

Product chemistry data submitted in support of a technical product, other manufacturing-use product, an experimental use permit, an import tolerance petition, or an end-use product produced from unregistered source ingredients, should be bound as a single study for each Guideline series (61, 62, and 63) for conventional pesticides, or for the equivalent subject range for biorational pesticides. The first of the three studies in a complete product chemistry submittal for a biochemical pesticide would cover Guidelines 151-10, 151-11, and 151-12; the second would cover Guidelines 151-13, 151-15, and 151-16; the third would cover Guideline 151-17. The first study for a microbial pesticide would cover Guidelines 151-20, 151-21, and 151-22; the second would cover Guidelines 151-23 and 151-25; the third would cover Guideline 151-26.

Note particularly that product chemistry studies are likely to contain Confidential Business Information as defined in FIFRA §10(d)(1)(A), (B), or (C), and if so must be handled as described in section D.3. of this notice.

c. Residue Chemistry Studies. Guidelines 171-4, 153-3, and 153-4 are extremely broad in scope; studies addressing residue chemistry requirements must thus be defined at a level below that of the Guideline code. The general principle, however, of limiting a study to the report of a single investigation still applies fully. Data should be treated as a single study and bound separately for each analytical method, each report of the nature of the residue in a single crop or animal species, and for each report of the magnitude of residues resulting from treatment of a single crop or from processing a single crop. When more than one commodity is derived from a single crop (such as beet tops and beet roots) residue data on all such commodities should be reported as a single study. When multiple field trials are associated with a single crop, all such trials should be reported as a single study.

D. Organization of Each Study Volume

Each complete study must include all applicable elements in the list below, in the order indicated. (Also see Page 17.) Several of these elements are further explained in the following paragraphs. Entries in the column headed "example" cite the page number of this notice where the element is illustrated.

Element	When Required	Example
Study Title Page	Always	Page 12
Statement of Data Confidentiality Claims	One of the two alternative forms of this statement is always required	Page 13
Certification of Good Laboratory Practice	If study reports laboratory work subject to GLP requirements	Page 16
Flagging statements	For certain toxicology studies (When flagging requirements are finalized.)	
Body of Study	Always - with an English language translation if required.	
Study Appendices	At submitter's option	
Cover Sheet to Confidential Attachment	If CBI is claimed under FIFRA §10(d)(1)(A), (B), or (C)	
CBI Attachment	If CBI is claimed under FIFRA §10(d)(1)(A), (B), or (C)	Page 15
Supplemental Statement of Data Confidentiality Claims	Only if confidentiality is claimed on a basis other than FIFRA §10(d)(1)(A), (B), or (C)	Page 14

D.1. Title Page

A title page is always required for each submitted study, published or unpublished. The title page must always be freely releasable to requestors; **DO NOT INCLUDE CBI ON THE TITLE PAGE.** An example of an acceptable title page is on page 12 of this notice. The following information must appear on the title page:

a. Study title. The study title should be as descriptive as possible It must clearly identify the substance(s) tested and correspond to the name of the data requirement as it appears in the Guidelines.

b. Data requirement addressed. Include on the title page the Guideline number(s) of the specific requirement(s) addressed by the study.

c. Author(s). Cite only individuals with primary intellectual responsibility for the content of the study. Identify them plainly as authors, to distinguish them from the performing laboratory, study sponsor, or other names that may also appear on the title page.

d. Study Date. The title page must include a single date for the study. If parts of the study were performed at different times, use only the date of the latest element in the study.

e. Performing Laboratory Identification. If the study reports work done by one or more laboratories, include on the title page the name and address of the performing laboratory or laboratories, and the laboratory's internal project number(s) for the work. Clearly distinguish the laboratory's project identifier from any other reference numbers provided by the study sponsor or submitter.

f. Supplemental Submissions. If the study is a commentary on or supplement to another previously submitted study, or if it responds to EPA questions raised with respect to an earlier study, include on the title page elements a. through d. for the previously submitted study, along with the EPA Master Record Identifier (MRID) or Accession number of the earlier study if you know these numbers. (Supplements submitted in the same submittal package as the primary study should be appended to and bound with the primary study. Do not include supplements to more than one study under a single title page).

g. Facts of Publication. If the study is a reprint of a published document, identity on the title page all relevant facts of publication, such as the journal title, volume, issue, inclusive page numbers, and publication date.

D.2. Statements of Data Confidentiality Claims Under FIFRA §10(d)(1).

Each submitted study must be accompanied by one of the two alternative forms of the statement of Data Confidentiality Claims specified in the proposed regulation in §158.33 (b) and (c) (See Attachment 3). These statements apply only to claims of data confidentiality based on FIFRA §10(d)(1)(A), (B), or (C). Use the appropriate alternative form of the statement either to assert a claim of §10(d)(1) data confidentiality (§158.33(b)) or to waive such a claim (§158.33(c)). In either case, the statement must be signed and dated, and must include the typed name and title of the official who signs it. Do not make CBI claims with respect to analytical methods associated with petitions for tolerances or emergency exemptions (see NOTE Pg 13).

D.3. Confidential Attachment

If the claim is made that a study includes confidential business information as defined by the criteria of FIFRA §10(D)(1)(A), (B), or (C) (as described in D.2. above) all such information must be excised from the body of the study and confined to a separate study-specific Confidential Attachment. Each passage of CBI so isolated must be identified by a reference number cited within the body of the study at the point from which the passage was excised (See Attachment 5).

The Confidential Attachment to a study must be identified by a cover sheet fully identifying the parent study, and must be clearly marked "Confidential Attachment." An appropriately annotated photocopy of the parent study title page may be used as this cover sheet. Paginate the Confidential Attachment separately from the body of the study, beginning with page 1 of X on the title page. Each passage confined to the Confidential Attachment must be associated with a specific cross reference to the page(s) in the main body of the study on which it is cited, and with a reference to the applicable passage(s) of FIFRA §10(d)(1) on which the confidentiality claim is based.

D.4. Supplemental Statement of Data Confidentiality Claims (See Attachment 4)

If you wish to make a claim of confidentiality for any portion of a submitted study other than described by FIFRA §10(d)(1)(A), (B), or (C), the following provisions apply:

- The specific information to which the claim applies must be clearly marked in the body of the study as subject to a claim of confidentiality.

- A Supplemental Statement of Data Confidentiality Claims must be submitted, identifying each passage claimed confidential and describing in detail the basis for the claim. A list of the points to address in such a statement is included in Attachment 4 on Pg 14.

- The Supplemental Statement of Data Confidentiality Claims must be signed and dated and must include the typed name and title of the official who signed it.

D.5. Good Laboratory Practice Compliance Statement

This statement is required if the study contains laboratory work subject to GLP requirements specified in 40 CFR 160. Samples of these statements are shown in Attachment 6.

E. Reference to Previously Submitted Data

DO NOT RESUBMIT A STUDY THAT HAS PREVIOUSLY BEEN SUBMITTED FOR ANOTHER PURPOSE unless EPA specifically requests it. A copy of the title page plus the MRID number (if known) is sufficient to allow us to retrieve the study immediately for review. This prevents duplicate entries in the Agency files, and saves you the cost of sending more copies of the study. References to previously submitted studies should not be included in the transmittal document, but should be incorporated into the statement of the method of support for the application.

F. Physical Format Requirements

All elements in the data submittal package must be on uniform 8 1/2 by 11 inch white paper, printed on one side only in black ink, with high contrast and good resolution. Bindings for individual studies must be secure, but easily removable to permit disassembly for microfilming. Check with EPA for special instructions before submitting data in any medium other than paper, such as film or magnetic media.

Please be particularly attentive to the following points:

- Do not include frayed or torn pages.
- Do not include carbon copies, or copies in other than black ink.
- Make sure that photocopies are clear, complete, and fully readable.
- Do not include oversize computer printouts or fold-out pages.
- Do not bind any documents with glue or binding tapes.
- Make sure that all pages of each study, including any attachments or appendices, are present and in correct sequence.

Number of Copies Required - All submittal packages except those associated with a Registration Standard or Special Review (See Part G below) must be provided ln three complete, identical copies. (The proposed regulations specified two copies; three are now being required to expedite and reduce the cost of processing data into the OPP Pesticide Document Management System and getting it into review.)

G. Special Requirements for Submitting Data to the Docket

Data submittal packages associated with a Registration Standard or Special Review must be provided in four copies, from one of which all material claimed as CBI has been excised. This fourth copy will become part of the public docket for the RS or SR case. If no claims of confidentiality are made for the study, the fourth copy should be identical to the other three. When portions of a study submitted in support of an RS or SR are claimed as CBI, the first three copies will include the CBI material as provided in section D of this notice. The following special preparation is required for the fourth copy.

- Remove the "Supplemental Statement of Data Confidentiality Claims".
- Remove the "Confidential Attachment".
- Excise from the body of the study any information you claim as confidential, even if it does not fall within the scope of FIFRA §10(d)(1)(A), (B), or (C). Do not close up or paraphrase text remaining after this excision.
- Mark the fourth copy plainly on both its cover and its title page with the phrase "Public Docket Material - contains no information claimed as confidential".

V. For Further Information

For further information contact John Carley, Chief, Information Services Branch, Program Management and Support Division, (703) 305-5240.

James W. Akerman
Acting Director,
Registration Division

Attachment 1. Sample Transmittal Document
Attachment 2. Sample Title Page for a Newly Submitted Study
Attachment 3. Statements of Data Confidentiality Claims
Attachment 4. Supplemental Statement of Data Confidentiality Claims
Attachment 5. Samples of Confidential Attachments
Attachment 6. Sample Good Laboratory Practice Statements
Attachment 7. Format Diagrams for Submittal Packages and Studies

ATTACHMENT 1

ELEMENTS TO BE INCLUDED IN THE TRANSMITTAL DOCUMENT*

1. Name and address of submitter (or all joint submitters**)

+Smith Chemical Corporation 1234 West Smith Street Cincinnati, OH 98765	-and-	Jones Chemical Company 5678 Wilson Blvd Covington, KY 56789

+Smith Chemical Corp will act as sole agent for all submitters.

2. Regulatory action in support of which this package is submitted

Use the EPA identification number (e.g. 359-EUP-67) if you know it. Otherwise describe the type of request (e.g. experimental use permit, data call-in - of xx-xx-xx date).

3. Transmittal date

4. List of submitted studies

Vol 1. Administrative materials - forms, previous correspondence with Project Managers, and so forth.

Vol 2. Title of first study in the submittal (Guideline No.)

Vol n Title of nth study in the submittal (Guideline No.)

* Applicants commonly provide this information in a transmittal letter. This remains an acceptable practice so long as all four elements are included.

* Indicate which of the joint submitters is empowered to act on behalf of all joint submitters in any matter concerning data compensation or subsequent use or release of the data.

Company Official: _____
Name Signature

Company Name: _____

Company Contact: _____ _____
Name Phone

ATTACHMENT 2

SAMPLE STUDY TITLE PAGE FOR A NEWLY SUBMITTED STUDY

Study Title

(Chemical name) - Magnitude of Residue on Corn

Data Requirement

Guideline 171-4

Author

John C. Davis

Study Completed On

January 5, 1979

Performing Laboratory

ABC Agricultural Laboratories
940 West Bay Drive
Wilmington, CA 39897

Laboratory Project ID

ABC 47-79

Page 1 of X
(X is the total number of pages in the study)

ATTACHMENT 3

STATEMENTS OF DATA CONFIDENTIALITY CLAIMS

1. No claim of confidentiality under FIFRA §10(d)(1)(A),(B), or (C).

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA 6§10(d)(1)(A), (B), or (C).

Company _____

Company Agent: _____ Typed Name _____ Date: _____

_____ Title _____ _____ Signature _____

2. Claim of confidentiality under FIFRA §10(d)(1)(A), (B), or (C).

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

Information claimed confidential on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C) has been removed to a confidential appendix, and is cited by cross-reference number in the body of the study.

Company: _____

Company Agent: _____ Typed Name _____ Date: _____

_____ Title _____ _____ Signature _____

NOTE: Applicants for permanent or temporary tolerances should note that it is OPP policy that no permanent tolerance, temporary tolerance, or request for an emergency exemption incorporating an analytical method, can be approved unless the applicant waives all claims of confidentiality for the analytical method. These analytical methods are published in the FDA Pesticide Analytical Methods Manual, and therefore cannot be claimed as confidential. OPP implements this policy by returning submitted analytical methods, for which confidentiality claims have been made, to the submitter, to obtain the confidentiality waiver before they can be processed.

ATTACHMENT 4

SUPPLEMENTAL STATEMENT OF DATA CONFIDENTIALITY CLAIMS

For any portion of a submitted study that is not described by FIFRA §10(d)(1)(A), (B), or (C), but for which you claim confidential treatment on another basis, the following information must be included within a Supplemental Statement of Data Confidentiality Claims:

- Identify specifically by page and line number(s) each portion of the study for which you claim confidentiality.
- Cite the reasons why the cited passage qualifies for confidential treatment.
- Indicate the length of time--until a specific date or event, or permanently--for which the information should be treated as confidential.
- Identify the measures taken to guard against undesired disclosure of this information.
- Describe the extent to which the information has been disclosed, and what precautions have been taken in connection with those disclosures.
- Enclose copies of any pertinent determinations of confidentiality made by EPA, other Federal agencies, of courts concerning this information.
- If you assert that disclosure of this information would be likely to result in substantial harmful effects to you, describe those harmful effects and explain why they should be viewed as substantial.
- If you assert that the information in voluntarily submitted, indicate whether you believe disclosure of this information might tend to lessen the availability to EPA of similar information in the future, and if so, how.

ATTACHMENT 5

EXAMPLES OF SEVERAL CONFIDENTIAL ATTACHMENTS

Example 1. (Confidential word or phrase that has been deleted from the study)

CROSS REFERENCE NUMBER 1 This cross reference number is used in the study in place of the following words or phrase at the indicated volume and page references.

DELETED WORDS OR PHRASE: Ethylene Glycol

PAGE	LINE	REASON FOR THE DELETION	FIFRA REFERENCE
6	14	Identity of Inert Ingredient	§10(d)(1)(C)
12	25	"	"
100	19	"	"

Example 2. (Confidential paragraph(s) that have been deleted from the study)

CROSS REFERENCE NUMBER 5 This cross reference number is used in the study in place of the following paragraph(s) at the indicated volume and page references.

DELETED PARAGRAPH(S):

(Reproduce the deleted paragraph(s) here)

PAGE	LINES	REASON FOR THE DELETION	FIFRA REFERENCE
20.	2-17	Description of the quality control process	§10(d)(1)(C)

Example 3. (Confidential pages that have been deleted from the study)

CROSS REFERENCE NUMBER 7 This cross reference number noted on a place-holder page is used in place of the following whole pages at the indicated volume and page references.

DELETED PAGE(S): are attached immediately behind this page.

PAGE	LINES	REASON FOR THE DELETION	FIFRA REFERENCE
20.	2-17	Description of the product manufacturing process	§10(d)(1)(A)

ATTACHMENT 6.

SAMPLE GOOD LABORATORY PRACTICE STATEMENTS

Example 1.

This study meets the requirements for 40 CFR Part 160

Submitter _____

Sponsor _____

Study Director _____

Example 2.

This study does not meet the requirements of 40 CFR Part 160, and differs in the following ways:

1._____

2._____

3._____

Submitter_____

Sponsor_____

Study Director_____

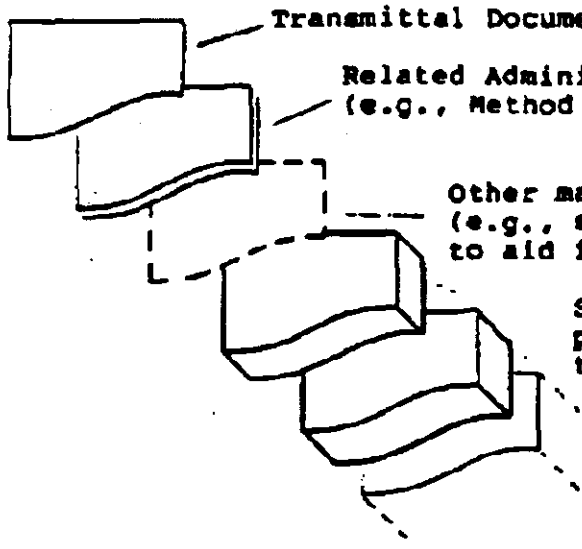
Example 3.

The submltter of this study was neither the sponsor of this study nor conducted it, and does not know whether it has been conducted in accordance with 40 CFR Part 160.

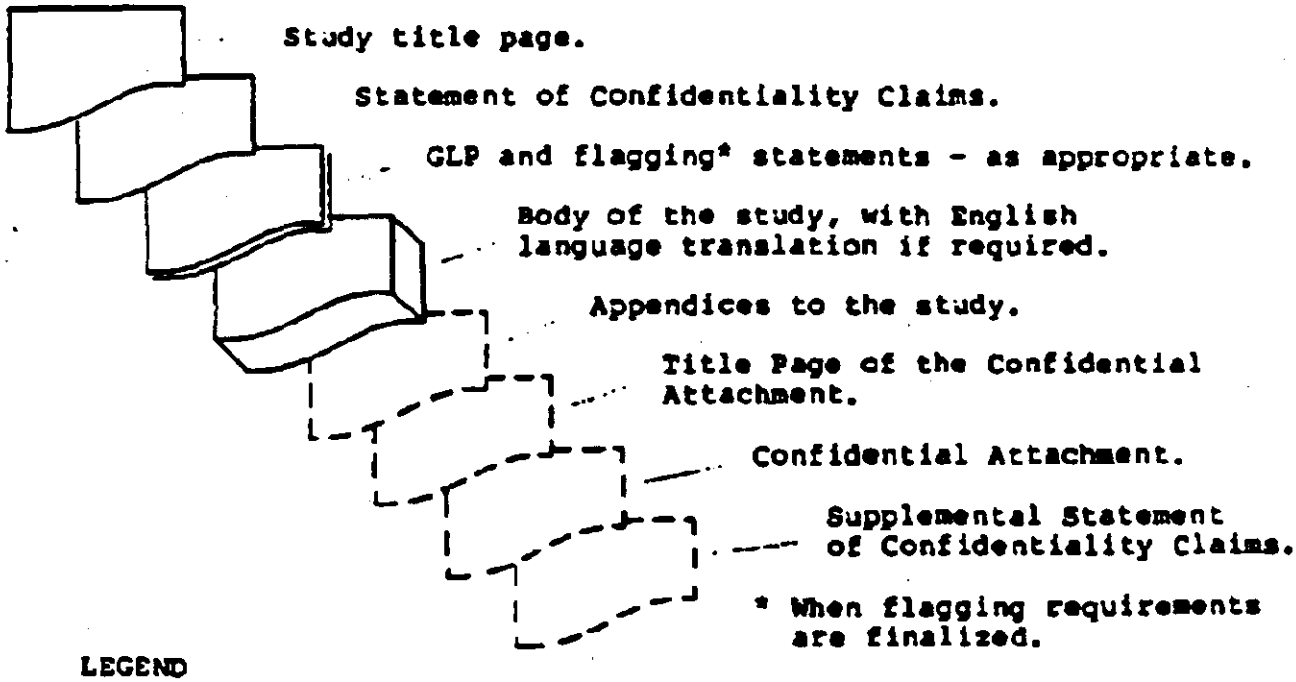
Submitter_____

ATTACHMENT 7.

FORMAT OF THE SUBMITTAL PACKAGE



FORMAT OF SUBMITTED STUDIES



LEGEND

17

PR Notice 91-2

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

PR NOTICE 91-2

NOTICE TO MANUFACTURERS, PRODUCERS, FORMULATORS, AND REGISTRANTS OF PESTICIDES

ATTENTION: Persons Responsible for Federal Registration of Pesticide Products.

SUBJECT: Accuracy of Stated Percentages for Ingredients Statement

I. PURPOSE:

The purpose of this notice is to clarify the Office of Pesticide Program's policy with respect to the statement of percentages in a pesticide's label's ingredient statement. Specifically, the amount (percent by weight) of ingredient(s) specified in the ingredient statement on the label must be stated as the nominal concentration of such ingredient(s), as that term is defined in 40 CFR 158.153(i). Accordingly, the Agency has established the nominal concentration as the only acceptable label claim for the amount of active ingredient in the product.

II. BACKGROUND

For some time the Agency has accepted two different methods of identifying on the label what percentage is claimed for the ingredient(s) contained in a pesticide. Some applicants claimed a percentage which represented a level between the upper and the lower certified limits. This was referred to as the nominal concentration. Other applicants claimed the lower limit as the percentage of the ingredient(s) that would be expected to be present in their product at the end of the product's shelf-life. Unfortunately, this led to a great deal of confusion among the regulated industry, the regulators, and the consumers as to exactly how much of a given ingredient was in a given product. The Agency has established the nominal concentration as the only acceptable label claim for the amount of active ingredient in the product.

Current regulations require that the percentage listed in the active ingredient statement be as precise as possible reflecting good manufacturing practices 40 CFR 156.10(g)(5). The

certified limits required for each active ingredient are intended to encompass any such "good manufacturing practice" variations 40 CFR 158.175(c)(3).
The upper and lower certified limits, which must be proposed in connection with a product's registration, represent the amounts of an ingredient that may legally be present 40 CFR 158.175. The lower certified limit is used as the enforceable lower limit for the product composition according to FIFRA section 12(a)(1)(C), while the nominal concentration appearing on the label would be the routinely achieved concentration used for calculation of dosages and dilutions.

The nominal concentration would in fact state the greatest degree of accuracy that is warranted with respect to actual product composition because the nominal concentration would be the amount of active ingredient typically found in the product.

It is important for registrants to note that certified limits for active ingredients are not considered to be trade secret information under FIFRA section 10(b). In this respect the certified limits will be routinely provided by EPA to States for enforcement purposes, since the nominal concentration appearing on the label may not represent the enforceable composition for purposes of section 12(a)(1)(C).

III. REQUIREMENTS

As described below under Unit V. " **COMPLIANCE SCHEDULE,"** all currently registered products as well as all applications for new registration must comply with this Notice by specifying the nominal concentration expressed as a percentage by weight as the label claim in the ingredient(s) statement and equivalence statements if applicable (e.g., elemental arsenic, metallic zinc, salt of an acid). In addition, the requirement for performing sample analyses of five or more representative samples must be fulfilled. Copies of the raw analytical data must be submitted with the nominal ingredient label claim. Further information about the analysis requirement may be found in the 40 CFR 158.170. All products are required to provide certified limits for each active, inert ingredient, impurities of toxicological significance(i.e., upper limit(s) only) and on a case by case basis as specified by EPA. These limits are to be **set based on representative sampling** and chemical analysis(i.e., quality control) of the product.

The format of the ingredient statement must conform to 40 CFR 156-Labeling Requirements For Pesticides and Devices.

After July 1, 1997, all pesticide ingredient StatementS must be changed to nominal concentration.

IV. PRODUCTS THAT REQUIRE EFFICACY DATA

All pesticides are required to be efficacious. Therefore, the certified lower limits may not be lower then the minimum level to achieve efficacy. This is extremely important for products which are intended to control pests which threaten the public health, e.g., certain antimicrobial and rodenticide products. Refer to 40 CFR 153.640.

In those cases where efficacy limits have been established, the Agency will not accept certified lower limits which are below that level for the shelf life of the product.

V. COMPLIANCE SCHEDULE

As described earlier, the purpose of this Notice is to make the registration process more uniform and more manageable for both the agency and the regulated community. It is the Agency's intention to implement the requirements of this notice as smoothly as possible so as not to disrupt or delay the Agency's high priority programs, i.e., reregistration, new chemical, or fast track (FIFRA section 3(c)(3)(B). Therefore, applicants/registrants are expected to comply with the requirements of this Notice as follows:

(1) Beginning July 1, 1991, all new product registrations submitted to the Agency are to comply with the requirements of this Notice.

(2) Registrants having products subject to reregistration under FIFRA section 4(a) are to comply with the requirements of this Notice when specific products are called in by the Agency under Phase V of the Reregistration Program.

(3) All other products/applications that are not subject to (1) and (2) above will have until July 1, 1997, to comply with this Notice. Such applications should note "Conversion to Nominal Concentrations on the application form. These types Or amendments will not be handled as "Fast Track" applications but will be handled as routine requests.

VI. FOR FURTHER INFORMATION

Contact Tyrone Aiken for information or questions concerning this notice on (703) 308-7031.

Anne E. Lindsay

Anne E. Lindsay, Director
Registration Division (H-7505

APPENDIX F

Generic Data Call-In

DATA CALL-IN NOTICE

CERTIFIED MAIL

Dear Sir or Madam:

This Notice requires you and other registrants of pesticide products containing the active ingredient(s) identified in Attachment A of this Notice, the Data Call-In Chemical Status Sheet, to submit certain data as noted herein to the U.S. Environmental Protection Agency (EPA, the Agency). These data are necessary to maintain the continued registration of your product(s) containing this active ingredient(s). Within 90 days after you receive this Notice you must respond as set forth in Section III below. Your response must state:

1. how you will comply with the requirements set forth in this Notice and its Attachments A through D; or,

2. why you believe you are exempt from the requirements listed in this Notice and in Attachment C, Requirements Status and Registrant's Response Form, (see section III-B); or,

3. why you believe EPA should not require your submission of data in the manner specified by this Notice (see section III-D).

If you do not respond to this Notice, or if you do not satisfy EPA that you will comply with its requirements or should be exempt or excused from doing so, then the registration of your product(s) subject to this Notice will be subject to suspension. We have provided a list of all of your products subject to this Notice in Attachment B, Data Call-In Response Form, as well as a list of all registrants who were sent this Notice (Attachment D).

The authority for this Notice is section 3(c)(2)(B) of the Federal Insecticide, Fungicide and Rodenticide Act as amended (FIFRA), 7 U.S.C. section 136a(c)(2)(B). Collection of this information is authorized under the Paperwork Reduction Act by OMB Approval No. 2070-0107 (expiration date 12-31-92).

This Notice is divided into six sections and five Attachments. The Notice itself contains information and instructions applicable to all Data Call-In Notices. The Attachments contain specific chemical information and instructions. The six sections of the Notice are:

Section I - Why You Are Receiving This Notice
Section II - Data Required By This Notice
Section III - Compliance With Requirements Of This Notice
Section IV - Consequences Of Failure To Comply With This Notice
Section V - Registrants' Obligation To Report Possible Unreasonable Adverse Effects
Section VI - Inquiries And Responses To This Notice

The Attachments to this Notice are:

Attachment A - Data Call-In Chemical Status Sheet
Attachment B - Data Call-In Response Form
Attachment C - Requirements Status And Registrant's Response Form
Attachment D - List Of All Registrants Sent This Data Call-In Notice

SECTION I. WHY YOU ARE RECEIVING THIS NOTICE

The Agency has reviewed existing data for this active ingredient(s) and reevaluated the data needed to support continued registration of the subject active ingredient(s). This reevaluation identified additional data necessary to assess the health and safety of the continued use of products containing this active ingredient(s). You have been sent this Notice because you have product(s) containing the subject active ingredient(s).

SECTION II. DATA REQUIRED BY THIS NOTICE

A. DATA REQUIRED

The data required by this Notice are specified in Attachment C, Requirements Status and Registrant's Response Form. Depending on the results of the studies required in this Notice, additional testing may be required.

B. SCHEDULE FOR SUBMISSION OF DATA

You are required to submit the data or otherwise satisfy the data requirements specified in Attachment C, Requirements Status and Registrant's Response Form, within the time frames provided.

C. TESTING PROTOCOL

All studies required under this Notice must be conducted in accordance with test standards outlined in the Pesticide Assessment Guidelines for those studies for which guidelines have been established.

These EPA Guidelines are available from the National Technical Information Service (NTIS), Attn: Order Desk, 5285 Port Royal Road, Springfield, Va 22161 (tel: 703-487-4650).

Protocols approved by the Organization for Economic Cooperation and Development (OECD) are also acceptable if the OECD-recommended test standards conform to those specified in the Pesticide Data Requirements regulation (40 CFR § 158.70). When using the OECD protocols, they should be modified as appropriate so that the data generated by the study will satisfy the requirements of 40 CFR § 158. Normally, the Agency will not extend deadlines for complying with data requirements when the studies were not conducted in accordance with acceptable standards. The OECD protocols are available from OECD, 1750 Pennsylvania Avenue N.W., Washington, D.C. 20006.

All new studies and proposed protocols submitted in response to this Data Call-In Notice must be in accordance with Good Laboratory Practices [40 CFR Part 160.3(a)(6)].

D. REGISTRANTS RECEIVING PREVIOUS SECTION 3(c)(2)(B) NOTICES ISSUED BY THE AGENCY

Unless otherwise noted herein, this Data Call-In does not in any way supersede or change the requirements of any previous Data Call-In(s), or any other agreements entered into with the Agency pertaining to such prior Notice. Registrants must comply with the requirements of all Notices to avoid issuance of a Notice of Intent to Suspend their affected products.

SECTION III. COMPLIANCE WITH REQUIREMENTS OF THIS NOTICE

A. SCHEDULE FOR RESPONDING TO THE AGENCY

The appropriate responses initially required by this Notice must be submitted to the ~~Agency within 90 days~~ after your receipt of this Notice. Failure to adequately respond to this Notice within 90 days of your receipt will be a basis for issuing a Notice of Intent to Suspend (NOIS) affecting your products. This and other bases for issuance of NOIS due to failure to comply with this Notice are presented in Section IV-A and IV-B.

B. OPTIONS FOR RESPONDING TO THE AGENCY

The options for responding to this Notice are: 1) voluntary cancellation, 2) delete use(s), (3) claim generic data exemption, (4) agree to satisfy the data requirements imposed by this Notice or (5) request a data waiver(s).

A discussion of how to respond if you chose the Voluntary Cancellation option, the Delete Use(s) option or the Generic Data Exemption option is presented below. A discussion of the various options available for satisfying the data requirements of this Notice is contained in Section III-C. A discussion of options relating to requests for data waivers is contained in Section III-D.

There are two forms that accompany this Notice of which, depending upon your response, one or both must be used in your response to the Agency. These forms are the Data-Call-In Response Form (Attachment B) and the Requirements Status and Registrant's Response Form (Attachment C). The Data Call-In Response Form must be submitted as part of every response to this Notice. Please note that the company's authorized representative is required to sign the first page of the Data Call-In Response Form and Requirements Status and Registrant's Response Form (if this form is required) and initial any subsequent pages. The forms contain separate detailed instructions on the response options. Do not alter the printed material. If you have questions or need assistance in preparing your response, call or write the contact person identified in Attachment A.

1. Voluntary Cancellation - You may avoid the requirements of this Notice by requesting voluntary cancellation of your product(s) containing the active ingredient(s) that is the subject of this Notice. If you wish to voluntarily cancel your product, you must submit a completed Data Call-In Response Form, indicating your election of this option. Voluntary cancellation is item number 5 on the Data Call-In Response Form. If you choose this option, this is the only form that you are required to complete.

If you choose to voluntarily cancel your product, further sale and distribution of your product after the effective date of cancellation must be in accordance with the Existing Stocks provisions of this Notice which are contained in Section IV-C.

2. Use Deletion - You may avoid the requirements of this Notice by eliminating the uses of your product to which the requirements apply. If you wish to ~~amend your~~ registration to delete uses, you must submit the Requirements Status and Registrant's Response Form, a completed application for amendment, a copy of your proposed amended labeling, and all other information required for processing the application. Use deletion is option number 7 on the Requirements Status and Registrant's Response Form. You must also complete a Data Call-In

Response Form by signing the certification, item number 8. Application forms for amending registrations may be obtained from the Registration Support and Emergency Response Branch, Registration Division, (703) 308-8358.

If you choose to delete the use(s) subject to this Notice or uses subject to specific data requirements, further sale, distribution, or use of your product after one year from the due date of your 90 day response, must bear an amended label.

3. Generic Data Exemption - Under section 3(c)(2)(D) of FIFRA, an applicant for registration of a product is exempt from the requirement to submit or cite generic data concerning an active ingredient(s) if the active ingredient(s) in the product is derived exclusively from purchased, registered pesticide products containing the active ingredient(s). EPA has concluded, as an exercise of its discretion, that it normally will not suspend the registration of a product which would qualify and continue to qualify for the generic data exemption in section 3(c)(2)(D) of FIFRA. To qualify, all of the following requirements must be met:

a. The active ingredient(s) in your registered product must be present solely because of incorporation of another registered product which contains the subject active ingredient(s) and is purchased from a source not connected with you; and,

b. every registrant who is the ultimate source of the active ingredient(s) in your product subject to this DCI must be in compliance with the requirements of this Notice and must remain in compliance; and

c. you must have provided to EPA an accurate and current "Confidential Statement of Formula" for each of your products to which this Notice applies.

To apply for the Generic Data Exemption you must submit a completed Data Call-In Response Form, Attachment B and all supporting documentation. The Generic Data Exemption is item number 6a on the Data Call-In Response Form. If you claim a generic data exemption you are not required to complete the Requirements Status and Registrant's Response Form. Generic Data Exemption cannot be selected as an option for product specific data.

If you are granted a Generic Data Exemption, you rely on the efforts of other persons to provide the Agency with the required data. If the registrant(s) who have committed to generate and submit the required data fail to take appropriate steps to meet the requirements or are no longer in compliance with this Data Call-In Notice, the Agency will consider that both they and you are not in compliance and will normally initiate proceedings to suspend the registrations

of both your and their product(s), unless you commit to submit and do submit the required data within the specified time. In such cases the Agency generally will not grant a time extension for submitting the data.

4. Satisfying the Data Requirements of this Notice - There are various options available to satisfy the data requirements of this Notice. These options are discussed in Section III-C of this Notice and comprise options 1 through 6 on the Requirements Status and Registrant's Response Form and option 6b and 7 on the Data Call-In Response Form. If you choose option 6b or 7, you must submit both forms as well as any other information/data pertaining to the option chosen to address the data requirement.

5. Request for Data Waivers. Data waivers are discussed in Section III-D of this Notice and are covered by options 8 and 9 on the Requirements Status and Registrant's Response Form. If you choose one of these options, you must submit both forms as well as any other information/data pertaining to the option chosen to address the data requirement.

C. SATISFYING THE DATA REQUIREMENTS OF THIS NOTICE

If you acknowledge on the Data Call-In Response Form that you agree to satisfy the data requirements (i.e. you select option 6b and/or 7), then you must select one of the six options on the Requirements Status and Registrant's Response Form related to data production for each data requirement. Your option selection should be entered under item number 9, "Registrant Response." The six options related to data production are the first six options discussed under item 9 in the instructions for completing the Requirements Status and Registrant's Response Form. These six options are listed immediately below with information in parentheses to guide registrants to additional instructions provided in this Section. The options are:

1. I will generate and submit data within the specified time frame (Developing Data),

2. I have entered into an agreement with one or more registrants to develop data jointly (Cost Sharing),

3. I have made offers to cost-share (Offers to Cost Share),

4. I am submitting an existing study that has not been submitted previously to the Agency by anyone (Submitting an Existing Study),

5. I am submitting or citing data to upgrade a study classified by EPA as partially acceptable and upgradeable (Upgrading a Study),

6. I am citing an existing study that EPA has classified as acceptable or an existing study that has been submitted but not reviewed by the Agency (Citing an Existing Study).

Option 1, Developing Data --

If you choose to develop the required data it must be in conformance with Agency deadlines and with other Agency requirements as referenced herein and in the attachments. All data generated and submitted must comply with the Good Laboratory Practice (GLP) rule (40 CFR Part 160), be conducted according to the Pesticide Assessment Guidelines (PAG), and be in conformance with the requirements of PR Notice 86-5. In addition, certain studies require Agency approval of test protocols in advance of study initiation. Those studies for which a protocol must be submitted have been identified in the Requirements Status and Registrant's Response Form and/or footnotes to the form. If you wish to use a protocol which differs from the options discussed in Section II-C of this Notice, you must submit a detailed description of the proposed protocol and your reason for wishing to use it. The Agency may choose to reject a protocol not specified in Section II-C. If the Agency rejects your protocol you will be notified in writing, however, you should be aware that rejection of a proposed protocol will not be a basis for extending the deadline for submission of data.

A progress report must be submitted for each study within 90 days from the date you are required to commit to generate or undertake some other means to address that study requirement, such as making an offer to cost-share or agreeing to share in the cost of developing that study. A 90-day progress report must be submitted for all studies. This 90-day progress report must include the date the study was or will be initiated and, for studies to be started within 12 months of commitment, the name and address of the laboratory(ies) or individuals who are or will be conducting the study.

In addition, if the time frame for submission of a final report is more than 1 year, interim reports must be submitted at 12 month intervals from the date you are required to commit to generate or otherwise address the requirement for the study. In addition to the other information specified in the preceding paragraph, at a minimum, a brief description of current activity on and the status of the study must be included as well as a full description of any problems encountered since the last progress report.

The time frames in the Requirements Status and Registrant's Response Form are the time frames that the Agency is allowing for the submission of completed study reports or protocols. The noted deadlines run from the date of the receipt of this Notice by the registrant. If the data are not submitted by the deadline, each registrant is subject to receipt of a Notice of Intent to Suspend the

affected registration(s).

If you cannot submit the data/reports to the Agency in the time required by this Notice and intend to seek additional time to meet the requirement(s), you must submit a request to the Agency which includes: (1) a detailed description of the expected difficulty and (2) a proposed schedule including alternative dates for meeting such requirements on a step-by-step basis. You must explain any technical or laboratory difficulties and provide documentation from the laboratory performing the testing. While EPA is considering your request, the original deadline remains. The Agency will respond to your request in writing. If EPA does not grant your request, the original deadline remains. Normally, extensions can be requested only in cases of extraordinary testing problems beyond the expectation or control of the registrant. Extensions will not be given in submitting the 90-day responses. Extensions will not be considered if the request for extension is not made in a timely fashion; in no event shall an extension request be considered if it is submitted at or after the lapse of the subject deadline.

Option 2, Agreement to Share in Cost to Develop Data --

If you choose to enter into an agreement to share in the cost of producing the required data but will not be submitting the data yourself, you must provide the name of the registrant who will be submitting the data. You must also provide EPA with documentary evidence that an agreement has been formed. Such evidence may be your letter offering to join in an agreement and the other registrant's acceptance of your offer, or a written statement by the parties that an agreement exists. The agreement to produce the data need not specify all of the terms of the final arrangement between the parties or the mechanism to resolve the terms. Section 3(c)(2)(B) provides that if the parties cannot resolve the terms of the agreement they may resolve their differences through binding arbitration.

Option 3, Offer to Share in the Cost of Data Development --

If you have made an offer to pay in an attempt to enter into an agreement or amend an existing agreement to meet the requirements of this Notice and have been unsuccessful, you may request EPA (by selecting this option) to exercise its discretion not to suspend your registration(s), although you do not comply with the data submission requirements of this Notice. EPA has determined that as a general policy, absent other relevant considerations, it will not suspend the registration of a product of a registrant who has in good faith sought and continues to seek to enter into a joint data development/cost sharing program, but the other registrant(s) developing the data has refused to accept your offer. To qualify for this option, you must submit documentation to the Agency proving that you have made an offer to another registrant (who has an obligation to submit

data) to share in the burden of developing that data. You must also submit to the Agency a completed EPA Form 8570-32, Certification of Offer to Cost Share in the Development of Data, Attachment E. In addition, you must demonstrate that the other registrant to whom the offer was made has not accepted your offer to enter into a cost sharing agreement by including a copy of your offer and proof of the other registrant's receipt of that offer (such as a certified mail receipt). Your offer must, in addition to anything else, offer to share in the burden of producing the data upon terms to be agreed or failing agreement to be bound by binding arbitration as provided by FIFRA section 3(c)(2)(B)(iii) and must not qualify this offer. The other registrant must also inform EPA of its election of an option to develop and submit the data required by this Notice by submitting a Data Call-In Response Form and a Requirements Status and Registrant's Response Form committing to develop and submit the data required by this Notice.

In order for you to avoid suspension under this option, you may not withdraw your offer to share in the burdens of developing the data. In addition, the other registrant must fulfill its commitment to develop and submit the data as required by this Notice. If the other registrant fails to develop the data or for some other reason is subject to suspension, your registration as well as that of the other registrant will normally be subject to initiation of suspension proceedings, unless you commit to submit, and do submit the required data in the specified time frame. In such cases, the Agency generally will not grant a time extension for submitting the data.

Option 4, Submitting an Existing Study --

If you choose to submit an existing study in response to this Notice, you must determine that the study satisfies the requirements imposed by this Notice. You may only submit a study that has not been previously submitted to the Agency or previously cited by anyone. Existing studies are studies which predate issuance of this Notice. Do not use this option if you are submitting data to upgrade a study. (See Option 5).

You should be aware that if the Agency determines that the study is not acceptable, the Agency will require you to comply with this Notice, normally without an extension of the required date of submission. The Agency may determine at any time that a study is not valid and needs to be repeated.

To meet the requirements of the DCI Notice for submitting an existing study, all of the following three criteria must be clearly met:

a. You must certify at the time that the existing study is submitted that the raw data and specimens from the study are available for audit and review and you must identify where they are available. This must be done in accordance with the requirements of the Good Laboratory Practice (GLP) regulation, 40 CFR Part 160. As stated in 40 CFR 160.3(7) " *raw data* means any laboratory worksheets, records, memoranda, notes, or exact copies thereof, that are the result of original observations and activities of a study and are necessary for the reconstruction and evaluation of the report of that study. In the event that exact transcripts of raw data have been prepared (e.g., tapes which have been transcribed verbatim, dated, and verified accurate by signature), the exact copy or exact transcript may be substituted for the original source as raw data. *Raw data* may include photographs, microfilm or microfiche copies, computer printouts, magnetic media, including dictated observations, and recorded data from automated instruments." The term "specimens", according to 40 CFR 160.3(7), means "any material derived from a test system for examination or analysis."

b. Health and safety studies completed after May 1984 must also contain all GLP-required quality assurance and quality control information, pursuant to the requirements of 40 CFR Part 160. Registrants must also certify at the time of submitting the existing study that such GLP information is available for post-May 1984 studies by including an appropriate statement on or attached to the study signed by an authorized official or representative of the registrant.

c. You must certify that each study fulfills the acceptance criteria for the Guideline relevant to the study provided in the FIFRA Accelerated Reregistration Phase 3 Technical Guidance and that the study has been conducted according to the Pesticide Assessment Guidelines (PAG) or meets the purpose of the PAG (both available from NTIS). A study not conducted according to the PAG may be submitted to the Agency for consideration if the registrant believes that the study clearly meets the purpose of the PAG. The registrant is referred to 40 CFR 158.70 which states the Agency's policy regarding acceptable protocols. If you wish to submit the study, you must, in addition to certifying that the purposes of the PAG are met by the study, clearly articulate the rationale why you believe the study meets the purpose of the PAG, including copies of any supporting information or data. It has been the Agency's experience that ~~studies completed~~ prior to January 1970 rarely satisfied the purpose of the PAG and that necessary raw data are usually not available for such studies.

If you submit an existing study, you must certify that the study meets all requirements of the criteria outlined above.

If EPA has previously reviewed a protocol for a study you are submitting, you must identify any action taken by the Agency on the protocol and must indicate, as part of your certification, the manner in which all Agency comments, concerns, or issues were addressed in the final protocol and study.

If you know of a study pertaining to any requirement in this Notice which does not meet the criteria outlined above but does contain factual information regarding unreasonable adverse effects, you must notify the Agency of such a study. If such a study is in the Agency's files, you need only cite it along with the notification. If not in the Agency's files, you must submit a summary and copies as required by PR Notice 86-5.

Option 5, Upgrading a Study --

If a study has been classified as partially acceptable and upgradeable, you may submit data to upgrade that study. The Agency will review the data submitted and determine if the requirement is satisfied. If the Agency decides the requirement is not satisfied, you may still be required to submit new data normally without any time extension. Deficient, but upgradeable studies will normally be classified as supplemental. However, it is important to note that not all studies classified as supplemental are upgradeable. If you have questions regarding the classification of a study or whether a study may be upgraded, call or write the contact person listed in Attachment A. If you submit data to upgrade an existing study you must satisfy or supply information to correct all deficiencies in the study identified by EPA. You must provide a clearly articulated rationale of how the deficiencies have been remedied or corrected and why the study should be rated as acceptable to EPA. Your submission must also specify the MRID number(s) of the study which you are attempting to upgrade and must be in conformance with PR Notice 86-5.

Do not submit additional data for the purpose of upgrading a study classified as unacceptable and determined by the Agency as not capable of being upgraded.

This option should also be used to cite data that has been previously submitted ~~to upgrade~~ a study, but has not yet been reviewed by the Agency. You must provide the MRID number of the data submission as well as the MRID number of the study being upgraded.

The criteria for submitting an existing study, as specified in Option 4

above, apply to all data submissions intended to upgrade studies. Additionally your submission of data intended to upgrade studies must be accompanied by a certification that you comply with each of those criteria as well as a certification regarding protocol compliance with Agency requirements.

Option 6. Citing Existing Studies --

If you choose to cite a study that has been previously submitted to EPA, that study must have been previously classified by EPA as acceptable or it must be a study which has not yet been reviewed by the Agency. Acceptable toxicology studies generally will have been classified as "core-guideline" or "core minimum." For ecological effects studies, the classification generally would be a rating of "core." For all other disciplines the classification would be "acceptable." With respect to any studies for which you wish to select this option you must provide the MRID number of the study you are citing and, if the study has been reviewed by the Agency, you must provide the Agency's classification of the study.

If you are citing a study of which you are not the original data submitter, you must submit a completed copy of EPA Form 8570-31, Certification with Respect to Data Compensation Requirements.

D. REQUESTS FOR DATA WAIVERS

There are two types of data waiver responses to this Notice. The first is a request for a low volume/minor use waiver and the second is a waiver request based on your belief that the data requirement(s) are inapplicable and do not apply to your product.

1. Low Volume/Minor Use Waiver -- Option 8 on the Requirements Status and Registrant's Response Form. Section 3(c)(2)(A) of FIFRA requires EPA to consider the appropriateness of requiring data for low volume, minor use pesticides. In implementing this provision EPA considers as low volume pesticides only those active ingredient(s) whose total production volume for all pesticide registrants is small. In determining whether to grant a low volume, minor use waiver the Agency will consider the extent, pattern and volume of use, the economic incentive to conduct the testing, the importance of the pesticide, and the exposure and risk from use of the pesticide. If an active ingredient(s) is used for both high volume and low volume uses, a low volume exemption will not be approved. If all uses of an active ingredient(s) are low volume and the combined ~~volumes for all uses~~ are also low, then an exemption may be granted, depending on review of other information outlined below. An exemption will not be granted if any registrant of the active ingredient(s) elects to conduct the testing. Any registrant receiving a low volume minor use waiver must remain within the sales figures in their forecast supporting the waiver request in order to remain qualified

for such waiver. If granted a waiver, a registrant will be required, as a condition of the waiver, to submit annual sales reports. The Agency will respond to requests for waivers in writing.

To apply for a low volume, minor use waiver, you must submit the following information, as applicable to your product(s), as part of your 90-day response to this Notice:

a. Total company sales (pounds and dollars) of all registered product(s) containing the active ingredient(s). If applicable to the active ingredient(s), include foreign sales for those products that are not registered in this country but are applied to sugar (cane or beet), coffee, bananas, cocoa, and other such crops. Present the above information by year for each of the past five years.

b. Provide an estimate of the sales (pounds and dollars) of the active ingredient(s) for each major use site. Present the above information by year for each of the past five years.

c. Total direct production cost of product(s) containing the active ingredient(s) by year for the past five years. Include information on raw material cost, direct labor cost, advertising, sales and marketing, and any other significant costs listed separately.

d. Total indirect production cost (e.g. plant overhead, amortized plant and equipment) charged to product(s) containing the active ingredient(s) by year for the past five years. Exclude all non-recurring costs that were directly related to the active ingredient(s), such as costs of initial registration and any data development.

e. A list of each data requirement for which you seek a waiver. Indicate the type of waiver sought and the estimated cost to you (listed separately for each data requirement and associated test) of conducting the testing needed to fulfill each of these data requirements.

f. A list of each data requirement for which you are not seeking any waiver and the estimated cost to you (listed separately for each data requirement and associated test) of conducting the testing needed to fulfill each of these data requirements.

g. For each of the next ten years, a year-by-year forecast of company sales (pounds and dollars) of the active ingredient(s), direct production costs of product(s) containing the active ingredient(s) (following the parameters in item c above), indirect production costs of product(s)

containing the active ingredient(s) (following the parameters in item d above), and costs of data development pertaining to the active ingredient(s).

h. A description of the importance and unique benefits of the active ingredient(s) to users. Discuss the use patterns and the effectiveness of the active ingredient(s) relative to registered alternative chemicals and non-chemical control strategies. Focus on benefits unique to the active ingredient(s), providing information that is as quantitative as possible. If you do not have quantitative data upon which to base your estimates, then present the reasoning used to derive your estimates. To assist the Agency in determining the degree of importance of the active ingredient(s) in terms of its benefits, you should provide information on any of the following factors, as applicable to your product(s):

(1) documentation of the usefulness of the active ingredient(s) in Integrated Pest Management, (b) description of the beneficial impacts on the environment of use of the active ingredient(s), as opposed to its registered alternatives, (c) information on the breakdown of the active ingredient(s) after use and on its persistence in the environment, and (d) description of its usefulness against a pest(s) of public health significance.

Failure to submit sufficient information for the Agency to make a determination regarding a request for a low volume minor use waiver will result in denial of the request for a waiver.

2. Request for Waiver of Data --Option 9 on the Requirements Status and Registrant's Response Form. This option may be used if you believe that a particular data requirement should not apply because the corresponding use is no longer registered or the requirement is inappropriate. You must submit a rationale explaining why you believe the data requirements should not apply. You must also submit the current label(s) of your product(s) and, if a current copy of your Confidential Statement of Formula is not already on file you must submit a current copy.

You will be informed of the Agency's decision in writing. If the Agency determines that the data requirements of this Notice do not apply to your product(s), you will not be required to supply the data pursuant to section 3(c)(2)(B). If EPA determines that the data are required for your product(s), you must choose a method of meeting the requirements of this Notice within the time frame provided by this Notice. Within 30 days of your receipt of the Agency's written decision, you must submit a revised Requirements Status and Registrant's Response Form indicating the option chosen.

IV. CONSEQUENCES OF FAILURE TO COMPLY WITH THIS NOTICE

A. NOTICE OF INTENT TO SUSPEND

The Agency may issue a Notice of Intent to Suspend products subject to this Notice due to failure by a registrant to comply with the requirements of this Data Call-In Notice, pursuant to FIFRA section 3(c)(2)(B). Events which may be the basis for issuance of a Notice of Intent to Suspend include, but are not limited to, the following:

1. Failure to respond as required by this Notice within 90 days of your receipt of this Notice.

2. Failure to submit on the required schedule an acceptable proposed or final protocol when such is required to be submitted to the Agency for review.

3. Failure to submit on the required schedule an adequate progress report on a study as required by this Notice.

4. Failure to submit on the required schedule acceptable data as required by this Notice.

5. Failure to take a required action or submit adequate information pertaining to any option chosen to address the data requirements (e.g., any required action or information pertaining to submission or citation of existing studies or offers, arrangements, or arbitration on the sharing of costs or the formation of Task Forces, failure to comply with the terms of an agreement or arbitration concerning joint data development or failure to comply with any terms of a data waiver).

6. Failure to submit supportable certifications as to the conditions of submitted studies, as required by Section III-C of this Notice.

7. Withdrawal of an offer to share in the cost of developing required data.

8. Failure of the registrant to whom you have tendered an offer to share in the cost of developing data and provided proof of the registrant's receipt of such offer, or failure of a registrant on whom you rely for a generic data exemption either to:

   a. inform EPA of intent to develop and submit the data required by this Notice on a Data Call-In Response Form and a Requirements Status and Registrant's Response Form; or,

   b. fulfill the commitment to develop and submit the data as required

by this Notice; or,

c. otherwise take appropriate steps to meet the requirements stated in this Notice, unless you commit to submit and do submit the required data in the specified time frame.

9. Failure to take any required or appropriate steps, not mentioned above, at any time following the issuance of this Notice.

B. BASIS FOR DETERMINATION THAT SUBMITTED STUDY IS UNACCEPTABLE

The Agency may determine that a study (even if submitted within the required time) is unacceptable and constitutes a basis for issuance of a Notice of Intent to Suspend. The grounds for suspension include, but are not limited to, failure to meet any of the following:

1. EPA requirements specified in the Data Call-In Notice or other documents incorporated by reference (including, as applicable, EPA Pesticide Assessment Guidelines, Data Reporting Guidelines, and GeneTox Health Effects Test Guidelines) regarding the design, conduct, and reporting of required studies. Such requirements include, but are not limited to, those relating to test material, test procedures, selection of species, number of animals, sex and distribution of animals, dose and effect levels to be tested or attained, duration of test, and, as applicable, Good Laboratory Practices.

2. EPA requirements regarding the submission of protocols, including the incorporation of any changes required by the Agency following review.

3. EPA requirements regarding the reporting of data, including the manner of reporting, the completeness of results, and the adequacy of any required supporting (or raw) data, including, but not limited to, requirements referenced or included in this Notice or contained in PR 86-5. All studies must be submitted in the form of a final report; a preliminary report will not be considered to fulfill the submission requirement.

C. EXISTING STOCKS OF SUSPENDED OR CANCELLED PRODUCTS

EPA has ~~statutory~~ authority to permit continued sale, distribution and use of existing stocks of a pesticide product which has been suspended or cancelled if doing so would be consistent with the purposes of the Federal Insecticide, Fungicide, and Rodenticide Act.

The Agency has determined that such disposition by registrants of existing stocks for a suspended registration when a section 3(c)(2)(B) data request is outstanding would generally not be consistent with the Act's purposes. Accordingly, the Agency anticipates granting registrants permission to sell, distribute, or use existing stocks of suspended product(s) only in exceptional circumstances. If you believe such disposition of existing stocks of your product(s) which may be suspended for failure to comply with this Notice should be permitted, you have the burden of clearly demonstrating to EPA that granting such permission would be consistent with the Act. You must also explain why an "existing stocks" provision is necessary, including a statement of the quantity of existing stocks and your estimate of the time required for their sale, distribution, and use. Unless you meet this burden the Agency will not consider any request pertaining to the continued sale, distribution, or use of your existing stocks after suspension.

If you request a voluntary cancellation of your product(s) as a response to this Notice and your product is in full compliance with all Agency requirements, you will have, under most circumstances, one year from the date your 90 day response to this Notice is due, to sell, distribute, or use existing stocks. Normally, the Agency will allow persons other than the registrant such as independent distributors, retailers and end users to sell, distribute or use such existing stocks until the stocks are exhausted. Any sale, distribution or use of stocks of voluntarily cancelled products containing an active ingredient(s) for which the Agency has particular risk concerns will be determined on case-by-case basis.

Requests for voluntary cancellation received <u>after</u> the 90 day response period required by this Notice will not result in the Agency granting any additional time to sell, distribute, or use existing stocks beyond a year from the date the 90 day response was due <u>unless</u> you demonstrate to the Agency that you are in full compliance with all Agency requirements, including the requirements of this Notice. For example, if you decide to voluntarily cancel your registration six months before a 3 year study is scheduled to be submitted, all progress reports and other information necessary to establish that you have been conducting the study in an acceptable and good faith manner must have been submitted to the Agency, before EPA will consider granting an existing stocks provision.

SECTION V. <u>REGISTRANTS' OBLIGATION TO REPORT POSSIBLE UNREASONABLE ADVERSE EFFECTS</u>

Registrants are reminded that FIFRA section 6(a)(2) states that if at any time after a pesticide is registered a registrant has additional factual information regarding unreasonable adverse effects on the environment by the pesticide, the registrant shall submit the information to the Agency. Registrants must notify the Agency of any factual information they have, from whatever source, including but not limited to interim or preliminary results of studies, regarding unreasonable adverse effects on man or the environment. This requirement continues as long as the products are registered by the Agency.

SECTION VI. INQUIRIES AND RESPONSES TO THIS NOTICE

If you have any questions regarding the requirements and procedures established by this Notice, call the contact person listed in Attachment A, the Data Call-In Chemical Status Sheet.

All responses to this Notice (other than voluntary cancellation requests and generic data exemption claims) must include a completed Data Call-In Response Form (Attachment B) and a completed Requirements Status and Registrant's Response Form (Attachment C) and any other documents required by this Notice, and should be submitted to the contact person identified in Attachment A. If the voluntary cancellation or generic data exemption option is chosen, only the Data Call-In Response Form need be submitted.

The Office of Compliance Monitoring (OCM) of the Office of Pesticides and Toxic Substances (OPTS), EPA, will be monitoring the data being generated in response to this Notice.

Sincerely yours,

Peter Caulkins

Peter P. Caulkins Ph.D., Acting Director
Special Review
and Reregistration Division

United States Environmental Protection Agency
Washington, D.C. 20460

REQUIREMENTS STATUS AND REGISTRANT'S RESPONSE

Form Approved
OMB No. 2070-0107
2070-0057
Approval Expires 03-31-96

INSTRUCTIONS: Please type or print in ink. Please read carefully the attached instructions and supply the information requested on this form.
Use additional sheet(s) if necessary

1. Company name and Address	2. Case # and Name 3150 Wood oils and gums Chemical # and Name 040505 Cedarwood oil	3. Date and Type of DCI GENERIC

4. Guideline Requirement Number	5. Study Title	PROTOCOL	Progress Reports 1	2	3	6. Use Pattern	7. Test Substance	8. Time Frame	9. Registrant Response
61-1	Chemical Identity					all	TGAI	8 mos.	
61-2(a)	Begin. mat. & mnfg. proc					all	TGAI	8 mos.	
61-2(b)	Discussion of Impurities					all	TGAI	8 mos.	
62-1	Preliminary Analysis					all	TGAI	8 mos.	
62-2	Certification of limits					all	TGAI	8 mos.	
62-3	Analytical Method					all	TGAI	8 mos.	

10. Certification

I certify that the statements made on this form and all attachments are true, accurate, and complete. I acknowledge that any knowingly false or misleading statement may be punishable by fine, imprisonment or both under applicable law.

Signature and Title of Company's Authorized Representative_____

11. Date

12. Name of Company Contact

13. Phone Number

Attachment A

Chemical Status Sheet

CEDARWOOD OIL: DATA CALL-IN CHEMICAL STATUS SHEET

INTRODUCTION

You have been sent this Generic Data Call-In Notice because you have a product containing cedarwood oil.

This Generic Data Call-In Chemical Status Sheet, contains an overview of data required by this notice, and a point of contact for inquiries pertaining to the reregistration of cedarwood oil. This attachment is to be used in conjunction with (1) the Generic Data Call-In Notice, (2) the Generic Data Call-In Response Form (Attachment B), (3) the Requirements Status and Registrant's Form (Attachment C), (4) a list of registrants receiving this DCI (Attachment D), (5) the EPA Acceptance Criteria (Attachment E), and (6) the Cost Share and Data Compensation Forms in replying to this cedarwood oil Generic Data Call-In (Attachment F). Instructions and guidance accompany each form.

DATA REQUIRED BY THIS NOTICE

The additional data requirements needed to complete the generic database for cedarwood oil are contained in the Requirements Status and Registrant's Response, Attachment C. The Agency has concluded that additional product chemistry data on cedarwood oil needed. These data are needed to fully complete the reregistration of all eligible cedarwood oil products.

INQUIRIES AND RESPONSES TO THIS NOTICE

If you have any questions regarding the generic data requirements and procedures established by this Notice, please contact Virginia Dietrich at (703) 308-8157. All responses to this Notice for the generic data requirements should be submitted to:

Virginia Dietrich, Chemical Review Manager
Accelerated Reregistration Branch
Special Review and Registration Division (7508W)
Office of Pesticide Programs
U.S. Environmental Protection Agency
Washington, D.C. 20460
RE: **Cedarwood Oil**

Attachment B

Generic DCI Response Forms (Form A) plus Instructions

SPECIFIC INSTRUCTIONS FOR THE DATA CALL-IN RESPONSE FORM

This Form is designed to be used to respond to call-ins for generic and product specific data for the purpose of reregistering pesticides under the Federal Insecticide Fungicide and Rodenticide Act. Fill out this form each time you are responding to a data call-in for which EPA has sent you the form entitled "Requirements Status and Registrant's Response."

Items 1-4 will have been preprinted on the form Items 5 through 7 must be completed by the registrant as appropriate Items 8 through 11 must be completed by the registrant before submitting a response to the Agency.

Public reporting burden for this collection of information is estimated to average 15 minutes per response, including time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding the burden estimate or any other aspect of this collection of information, including suggesting for reducing this burden, to Chief, Information Policy Branch, PM-223, U S Environmental Protection Agency, 401 M St , S W , Washington, D C 20460; and to the Office of Management and Budget, Paperwork Reduction Project 2070-0107, Washington, D C 20503

INSTRUCTIONS

Item 1 — This item identifies your company name, number and address.

Item 2 — This item identifies the ease number, ease name, EPA chemical number and chemical name.

Item 3 — This item identifies the date and type of data call-in.

Item 4 — This item identifies the EPA product registrations relevant to the data call-in. Please note that you are also responsible for informing the Agency of your response regarding any product that you believe may be covered by this data call-in but that is not listed by the Agency in Item 4. You must bring any such apparent omission to the Agency's attention within the period required for submission of this response form.

Item 5 — Cheek this item for each product registration you wish to cancel voluntarily. If a registration number is listed for a product for which you previously requested voluntary cancellation, indicate in Item 5 the date of that request. You do not need to complete any item on the Requirements Status and Registrant's Response Form for any product that is voluntarily cancelled.

Item 6a — Check this item if this data call-in is for generic data as indicated in Item 3 and if you are eligible for a Generic Data Exemption for the chemical listed in Item

2 and used in the subject product. By electing this exemption, you agree to the terms and conditions of a Generic Data Exemption as explained in the Data Call-In Notice.

If you are eligible for or claim a Generic Data Exemption, enter the EPA registration Number of each registered source of that active ingredient that you use in your product.

Typically, if you purchase an EPA-registered product from one or more other producers (who, with respect to the incorporated product, are in compliance with this and-any other outstanding Data Call-In Notice), and incorporate that product into all your products, you may complete this item for all products listed on this form If, however, you produce the active ingredient yourself, or use any unregistered product (regardless of the fact that some of your sources are registered), you may not claim a Generic Data Exemption and you may not select this item.

Item 6b — Check this Item if the data call-in is a generic data call-in as indicated in Item 3 and if you are agreeing to satisfy the generic data requirements of this data call-in. Attach the Requirements Status and Registrant's Response Form that lndicates how you will satisfy those requirements.

Item 7a — Check this item only if this call-in is a data call-in as indicated in Item 3 for a manufacturing use product (MUP), and if your product is a manufacturing use product for which you agree to supply product-specific data. Attach the Requirements Status and Registrants' Response Form that indicates how you will satisfy those requirements.

Item 7b — Check this item only if this call-in is a data call-in for an end use product (EUP) as indicated in Item 3 and if your product is an end use product for which you agree to supply product-specific data. Attach the Requirements Status and Registrant's Response Form that indicates how you will satisfy those requirements.

Item 8 — This certification statement must be signed by an authorized representative of your company and the person signing must include his/her title. Additional pages used in your response must be initialled and dated in the space provided for the certification.

Item 9 — Enter the date of signature.

Item 10 — Enter the name of the person EPA should contact with questions regarding your response.

Item 11 Enter the phone number of your company contact.

Attachment C

Requirements Status and Registrants' Response Forms (Form B) plus Instructions

SPECIFIC INSTRUCTIONS FOR COMPLETING THE REQUIREMENTS STATUS AND REGISTRANTS RESPONSE FORM

Generic Data

This form is designed to be used for registrants to respond to call-in- for generic and product-specific data as part of EPA's reregistration program under the Federal Insecticide Fungicide and Rodenticide Act. Although the form is the same for both product specific and generic data, instructions for completing the forms differ slightly. Specifically, options for satisfying product specific data requirements do not include (1) deletion of uses or (2) request for a low volume/minor use waiver. These instructions are for completion of generic data requirements.

EPA has developed this form individually for each data call-in addressed to each registrant, and has preprinted this form with a number of items. DO NOT use this form for any other active ingredient.

Items 1 through 8 (inclusive) will have been preprinted on the form. You must complete all other items on this form by typing or printing legibly.

Public reporting burden for this collection of information is estimated to average 30 minutes per response, including time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding the burden estimate or any other aspect of this collection of information, including suggesting for reducing this burden, to Chief, Information Policy Branch, PM-223, U.S. Environmental Protection Agency, 401 M St., S.W., Washington, D.C. 20460; and to the Office of Management and Budget, Paperwork Reduction Project 2070-0107, Washington, D.C. 20503.

INSTRUCTIONS

Item 1. This item identifies your company name, number, and address.

Item 2. This item identifies the case number, case name, EPA chemical number and chemical name.

Item 3. This item identifies the date and type of data call-in.

Item 4. This item identifies the guideline reference numbers of studies required to support the ~~product~~(s) being reregistered. These guidelines, in addition to requirements specified in the Data Call-In Notice, govern the conduct of the required studies.

Item 5. This item identifies the study title associated with the guideline reference

number and whether protocols and 1, 2, or 3-year progress reports are required to be submitted in connection with the study. As noted in Section III of the Data Call-In Notice, 90-day progress reports are required for all studies.

If an asterisk appears in Item 5, EPA has attached information relevant to this guideline reference number to the Requirements Status and Registrant's Response Form.

Item 6. This item identifies the code associated with the use pattern of the pesticide. A brief description of each code follows:

A.	Terrestrial food
B.	Terrestrial feed
C.	Terrestrial non-food
D.	Aquatic food
E.	Aquatic non-food outdoor
F.	Aquatic non-food industrial
G.	Aquatic non-food residential
H.	Greenhouse food
I.	Greenhouse non-food crop
J.	Forestry
K.	Residential
L.	Indoor food
M.	Indoor non-food
N.	Indoor medical
O.	Indoor residential

Item 7. This item identifies the code assigned to the substance that must be used for testing. A brief description of each code follows.

EP	End-Use Product
MP	Manufacturing-Use Product
MP/TGAI	Manufacturing-Use Product and Technical Grade Active Ingredient
PAI	Pure Active Ingredient
PAI/M	Pure Active Ingredient and Metabolites
PAI/PAIRA	Pure Active Ingredient or Pure Active Ingredient Radiolabelled
PAIRA	Pure Active Ingredient Radiolabelled
PAIRA/M	Pure Active Ingredient Radiolabelled and Metabolites
PAIRA/PM	Pure Active Ingredient Radiolabelled and Plant Metabolites
TEP	Typical End-Use Product
TEP _ *	Typical End-Use Product, Percent Active Ingredient

	Specified
TEP/MET	Typical End-Use Product and Metabolites
TEP/PAI/M	Typical End-Use Product or Pure Active Ingredient and Metabolites
TGAI/PAIRA	Technical Grade Active Ingredient or Pure Active Ingredient Radiolabelled
TGAI	Technical Grade Active Ingredient
TGAI/TEP	Technical Grade Active Ingredient or Typical End-Use Product
TGAI/PAI	Technical Grade Active Ingredient or Pure Active Ingredient
MET	Metabolites
IMP	Impurities
DEGR	Degradates

*See: guideline comment

Item 8. This item identifies the time frame allowed for submission of the study or protocol identified in item 2. The time frame runs from the date **of your** receipt of the Data Call-In Notice.

Item 9. Enter the appropriate Response Code or Codes to show how you intend to comply with each data requirement. Brief descriptions of each code follow. The Data Call-In Notice contains a fuller description of each of these options.

1. (Developing Data) I will conduct a new study and submit it within the time frames specified in item 8 above. By indicating that I have chosen this option, I certify that I will comply with all the requirements pertaining to the conditions for submittal of this study as outlined in the Data Call-In Notice and that I will provide the protocol and progress reports required in item 5 above.

2. (Agreement to Cost Share) I have entered into an agreement with one or more registrants to develop data jointly. By indicating that I have chosen this option, I certify that I will comply with all the requirements pertaining to sharing in the cost of developing data as outlined in the Data Call-In Notice.

3. (Offer to Cost Share) I have made an offer to enter into an agreement ~~with one or more~~ registrants to develop data jointly. I am submitting a copy of the form "Certification of Offer to Cost Share in the Development of Data" that describes this offer/agreement. By indicating that I have chosen this option, I certify that I will comply with all the requirements pertaining to making an offer to share in the

cost of developing data as outlined in the Data Call-In Notice.

4. (Submitting Existing Data) I am submitting an existing study that has never before been submitted to EPA. By indicating that I have chosen this option, I certify that this study meets all the requirements pertaining to the conditions for submittal of existing data outlined in the Data Call-In Notice and I have attached the needed supporting information along with this response.

5. (Upgrading a Study) I am submitting or citing data to upgrade a study that EPA has classified as partially acceptable and potentially upgradeable. By indicating that I have chosen this option, I certify that I have met all the requirements pertaining to the conditions for submitting or citing existing data to upgrade a study described in the Data Call-In Notice. I am indicating on attached correspondence the Master Record Identification Number (MRID) that EPA has assigned to the data that I am citing as well as the MRID of the study I am attempting to upgrade.

6. (Citing a Study) I am citing an existing study that has been previously classified by EPA as acceptable, core, core minimum, or a study that has not yet been reviewed by the Agency. I am providing the Agency's classification of the study.

7. (Deleting Uses) I am attaching an application for amendment to my registration deleting the uses for which the data are required.

8. (Low Volume/Minor Use Waiver Request) I have read the statements concerning low volume-minor use data waivers in the Data Call-In Notice and I request a low-volume minor use waiver of the data requirement. I am attaching a detailed justification to support this waiver request including, among other things, all information required to support the request. I understand that, unless modified by the Agency in writing, the data requirement as stated in the Notice governs.

9. (Request for Waiver of Data) I have read the statements concerning data waivers other than low volume minor-use data waivers in the Data Call-In Notice and I request a waiver of the data requirement. I am attaching an identification of the basis for this waiver and a detailed justification to support this waiver request. The justification includes, among other things, all information required to support the request. I understand that, unless modified by the Agency in writing, the data requirement as stated in the Notice governs.

Item 10. This item must be signed by an authorized representative of your company. The person signing must include his/her title, and must initial and date all other pages of this form.

Item 11. Enter the date of signature.

Item 12. Enter the name of the person EPA should contact with questions regarding your response.

Item 13. Enter the phone number of your company contact.

Attachment D

List of Registrants Sent This DCI

APPENDIX G

Product Specific Data Call-In

DATA CALL-IN NOTICE

CERTIFIED MAIL

Dear Sir or Madam:

This Notice requires you and other registrants of pesticide products containing the active ingredient identified in Attachment A of this Notice, the Data Call-In Chemical Status Sheet, to submit certain product specific data as noted herein to the U.S. Environmental Protection Agency (EPA, the Agency). These data are necessary to maintain the continued registration of your product(s) containing this active ingredient. Within 90 days after you receive this Notice you must respond as set forth in Section III below. Your response must state:

1. How you will comply with the requirements set forth in this Notice and its Attachments A through G; or

2. Why you believe you are exempt from the requirements listed in this Notice and in Attachment C, Requirements Status and Registrant's Response Form, (see section III-B); or

3. Why you believe EPA should not require your submission of product specific data in the manner specified by this Notice (see section III-D).

If you do not respond to this Notice, or if you do not satisfy EPA that you will comply with its requirements or should be exempt or excused from doing so, then the registration of your product(s) subject to this Notice will be subject to suspension. We have provided a list of all of your products subject to this Notice in Attachment B, Data Call-In Response Form, as well as a list of all registrants who were sent this Notice (Attachment F).

The authority for this Notice is section 3(c)(2)(B) of the Federal Insecticide, Fungicide and Rodenticide Act as amended (FIFRA), 7 U.S.C. section 136a(c)(2)(B). Collection of this information is authorized under the Paperwork Reduction Act by OMB Approval No. 2070-0107 (expiration date 12-31-92).

This Notice is divided into six sections and seven Attachments. The Notice itself contains information and instructions applicable to all Data Call-In Notices. The Attachments contain specific chemical information and instructions. The six sections of the Notice are:

Section I - Why You Are Receiving This Notice
Section II - Data Required By This Notice
Section III- Compliance With Requirements Of This Notice
Section IV - Consequences Of Failure To Comply With This Notice
Section V - Registrants' Obligation To Report Possible Unreasonable Adverse Effects
Section VI - Inquiries And Responses To This Notice

The Attachments to this Notice are:

A - Data Call-In Chemical Status Sheet
B - Product-Specific Data Call-In Response Form
C - Requirements Status and Registrant's Response Form
D - EPA Grouping of End-Use Products for Meeting Acute Toxicology Data Requirements for Reregistration
E - EPA Acceptance Criteria
F - List of Registrants Receiving This Notice
G - Cost Share and Data Compensation Forms, and Product Specific Data Report Form

SECTION I. WHY YOU ARE RECEIVING THIS NOTICE

The Agency has reviewed existing data for this active ingredient and reevaluated the data needed to support continued registration of the subject active ingredient. The Agency has concluded that the only additional data necessary are product specific data. No additional generic data requirements are being imposed. You have been sent this Notice because you have product(s) containing the subject active ingredient.

SECTION II. DATA REQUIRED BY THIS NOTICE

II-A. DATA REQUIRED

The product specific data required by this Notice are specified in Attachment C, Requirements Status and Registrant's Response Form. Depending on the results of the studies required in this Notice, additional testing may be required.

II-B. SCHEDULE FOR SUBMISSION OF DATA

You are required to submit the data or otherwise satisfy the data requirements specified in Attachment C, Requirements Status and Registrant's Response Form, within the time frames provided.

II-C. TESTING PROTOCOL

All studies required under this Notice must be conducted in accordance with test standards outlined in the Pesticide Assessment Guidelines for those studies for which guidelines have been established.

These EPA Guidelines are available from the National Technical Information Service (NTIS), Attn: Order Desk, 5285 Port Royal Road, Springfield, Va 22161 (tel: 703-487-4650).

Protocols approved by the Organization for Economic Cooperation and Development (OECD) are also acceptable if the OECD-recommended test standards conform to those specified in the Pesticide Data Requirements regulation (40 CFR § 158.70). When using the OECD protocols, they should be modified as appropriate so that the data generated by the study will satisfy the requirements of 40 CFR § 158. Normally, the Agency will not extend deadlines for complying with data requirements when the studies were not conducted in accordance with acceptable standards. The OECD protocols are available from OECD, 1750 Pennsylvania Avenue N.W., Washington, D.C. 20006.

All new studies and proposed protocols submitted in response to this Data Call-In Notice must be in accordance with Good Laboratory Practices [40 CFR Part 160.3(a)(6)].

II-D. REGISTRANTS RECEIVING PREVIOUS SECTION 3(c)(2)(B) NOTICES ISSUED BY THE AGENCY

Unless otherwise noted herein, this Data Call-In does not in any way supersede or change the requirements of any previous Data Call-In(s), or any other agreements entered into with the Agency pertaining to such prior Notice. Registrants must comply with the requirements of all Notices to avoid issuance of a Notice of Intent to Suspend their affected products.

SECTION III. COMPLIANCE WITH REQUIREMENTS OF THIS NOTICE

III-A. SCHEDULE FOR RESPONDING TO THE AGENCY

The appropriate responses initially required by this Notice for product specific data must be submitted to the Agency within 90 days after your receipt of this Notice. Failure to adequately respond to this Notice within 90 days of your receipt will be a basis for issuing a Notice of Intent to Suspend (NOIS) affecting your products. This and other bases for issuance

of NOIS due to failure to comply with this Notice are presented in Section IV-A and IV-B.

III-B. OPTIONS FOR RESPONDING TO THE AGENCY

The options for responding to this Notice for product specific data are: (a) voluntary cancellation, (b) agree to satisfy the product specific data requirements imposed by this notice or (c) request a data waiver(s).

A discussion of how to respond if you chose the Voluntary Cancellation option is presented below. A discussion of the various options available for satisfying the product specific data requirements of this Notice is contained in Section III-C. A discussion of options relating to requests for data waivers is contained in Section III-D.

There are two forms that accompany this Notice of which, depending upon your response, one or both must be used in your response to the Agency. These forms are the Data-Call-In Response Form, and the Requirements Status and Registrant's Response Form, Attachment B and Attachment C. The Data Call-In Response Form must be submitted as part of every response to this Notice. In addition, one copy of the Requirements Status and Registrant's Response Form must be submitted for each product listed on the Data Call-In Response Form unless the voluntary cancellation option is selected or unless the product is identical to another (refer to the instructions for completing the Data Call-In Response Form in Attachment B). Please note that the company's authorized representative is required to sign the first page of the Data Call-In Response Form and Requirements Status and Registrant's Response Form (if this form is required) and initial any subsequent pages. The forms contain separate detailed instructions on the response options. Do not alter the printed material. If you have questions or need assistance in preparing your response, call or write the contact person(s) identified in Attachment A.

1. Voluntary Cancellation - You may avoid the requirements of this Notice by requesting voluntary cancellation of your product(s) containing the active ingredient that is the subject of this Notice. If you wish to voluntarily cancel your product, you must submit a completed Data Call-In Response Form, indicating your election of this option. Voluntary cancellation is item number 5 on the Data Call-In Response Form. If you choose this option, this is the only form that you are required to complete.

If you chose to voluntarily cancel your product, further sale and distribution of your product after the effective date of cancellation must be in accordance with the Existing Stocks provisions of this Notice which are contained in Section IV-C.

2. Satisfying the Product Specific Data Requirements of this Notice There are various options available to satisfy the product specific data requirements of this Notice. These options are discussed in Section III-C of this Notice and comprise options 1 through 6 on the

Requirements Status and Registrant's Response Form and item numbers 7a and 7b on the Data Call-In Response Form. Deletion of a use(s) and the low volume/minor use option are not valid options for fulfilling product specific data requirements.

3. Request for Product Specific Data Waivers. Waivers for product specific data are discussed in Section III-D of this Notice and are covered by option 7 on the Requirements Status and Registrant's Response Form. If you choose one of these options, you must submit both forms as well as any other information/data pertaining to the option chosen to address the data requirement.

III-C SATISFYING THE DATA REQUIREMENTS OF THIS NOTICE

If you acknowledge on the Data Call-In Response Form that you agree to satisfy the product specific data requirements (i.e. you select item number 7a or 7b), then you must select one of the six options on the Requirements Status and Registrant's Response Form related to data production for each data requirement. Your option selection should be entered under item number 9, "Registrant Response." The six options related to data production are the first six options discussed under item 9 in the instructions for completing the Requirements Status and Registrant's Response Form. These six options are listed immediately below with information in parentheses to guide registrants to additional instructions provided in this Section. The options are:

(1) I will generate and submit data within the specified time frame (Developing Data)
(2) I have entered into an agreement with one or more registrants to develop data jointly (Cost Sharing)
(3) I have made offers to cost-share (Offers to Cost Share)
(4) I am submitting an existing study that has not been submitted previously to the Agency by anyone (Submitting an Existing Study)
(5) I am submitting or citing data to upgrade a study classified by EPA as partially acceptable and upgradeable (Upgrading a Study)
(6) I am citing an existing study that EPA has classified as acceptable or an existing study that has been submitted but not reviewed by the Agency (Citing an Existing Study)

Option 1, Developing Data -- If you choose to develop the required data it must be in conformance with Agency deadlines and with other Agency requirements as referenced herein and in the attachments. All data generated and submitted must comply with the Good Laboratory Practice (GLP) rule (40 CFR Part 160), be conducted according to the Pesticide Assessment Guidelines (PAG), and be in conformance with the requirements of PR Notice 86-5.

The time frames in the Requirements Status and Registrant's Response Form are the time frames that the Agency is allowing for the submission of completed study reports. The noted deadlines run from the date of the receipt of this Notice by the registrant. If the data are not

submitted by the deadline, each registrant is subject to receipt of a Notice of Intent to Suspend the affected registration(s).

If you cannot submit the data/reports to the Agency in the time required by this Notice and intend to seek additional time to meet the requirements(s), you must submit a request to the Agency which includes: (1) a detailed description of the expected difficulty and (2) a proposed schedule including alternative dates for meeting such requirements on a step-by-step basis. You must explain any technical or laboratory difficulties and provide documentation from the laboratory performing the testing. While EPA is considering your request, the original deadline remains. The Agency will respond to your request in writing. If EPA does not grant your request, the original deadline remains. Normally, extensions can be requested only in cases of extraordinary testing problems beyond the expectation or control of the registrant. Extensions will not be given in submitting the 90-day responses. Extensions will not be considered if the request for extension is not made in a timely fashion; in no event shall an extension request be considered if it is submitted at or after the lapse of the subject deadline.

Option 2, Agreement to Share in Cost to Develop Data -- Registrants may only choose this option for acute toxicity data and certain efficacy data and only if EPA has indicated in the attached data tables that your product and at least one other product are similar for purposes of depending on the same data. If this is the case, data may be generated for just one of the products in the group. The registration number of the product for which data will be submitted must be noted in the agreement to cost share by the registrant selecting this option. If you choose to enter into an agreement to share in the cost of producing the required data but will not be submitting the data yourself, you must provide the name of the registrant who will be submitting the data. You must also provide EPA with documentary evidence that an agreement has been formed. Such evidence may be your letter offering to join in an agreement and the other registrant's acceptance of your offer, or a written statement by the parties that an agreement exists. The agreement to produce the data need not specify all of the terms of the final arrangement between the parties or the mechanism to resolve the terms. Section 3(c)(2)(B) provides that if the parties cannot resolve the terms of the agreement they may resolve their differences through binding arbitration.

Option 3, Offer to Share in the Cost of Data Development -- This option only applies to acute toxicity and certain efficacy data as described in option 2 above. If you have made an offer to pay in an attempt to enter into an agreement or amend an existing agreement to meet the requirements of this Notice and have been unsuccessful, you may request EPA (by selecting this option) to exercise its discretion not to suspend your registration(s), although you do not comply with the data submission requirements of this Notice. EPA has determined that as a general policy, absent other relevant considerations, it will not suspend the registration of a product of a registrant who has in good faith sought and continues to seek to enter into a joint data development/cost sharing program, but the other registrant(s) developing the data has refused to accept your offer. To qualify for this option, you must submit documentation to the Agency proving ~~that you have~~ made an offer to another registrant (who has an obligation to submit data) to share in the burden of developing that data. You must also submit to the Agency a completed EPA Form 8570-32, Certification of Offer to Cost Share in the Development of Data, Attachment G. In addition, you must demonstrate that the other registrant to whom the offer was made has not accepted your offer to enter into a cost sharing

agreement by including a copy of your offer and proof of the other registrant's receipt of that offer (such as a certified mail receipt). Your offer must, in addition to anything else, offer to share in the burden of producing the data upon terms to be agreed or failing agreement to be bound by binding arbitration as provided by FIFRA section 3(c)(2)(B)(iii) and must not qualify this offer. The other registrant must also inform EPA of its election of an option to develop and submit the data required by this Notice by submitting a Data Call-In Response Form and a Requirements Status and Registrant's Response Form committing to develop and submit the data required by this Notice.

In order for you to avoid suspension under this option, you may not withdraw your offer to share in the burdens of developing the data. In addition, the other registrant must fulfill its commitment to develop and submit the data as required by this Notice. If the other registrant fails to develop the data or for some other reason is subject to suspension, your registration as well as that of the other registrant will normally be subject to initiation of suspension proceedings, unless you commit to submit, and do submit the required data in the specified time frame. In such cases, the Agency generally will not grant a time extension for submitting the data.

Option 4, Submitting an Existing Study -- If you choose to submit an existing study in response to this Notice, you must determine that the study satisfies the requirements imposed by this Notice. You may only submit a study that has not been previously submitted to the Agency or previously cited by anyone. Existing studies are studies which predate issuance of this Notice. Do not use this option if you are submitting data to upgrade a study. (See Option 5).

You should be aware that if the Agency determines that the study is not acceptable, the Agency will require you to comply with this Notice, normally without an extension of the required date of submission. The Agency may determine at any time that a study is not valid and needs to be repeated.

To meet the requirements of the DCI Notice for submitting an existing study, all of the following three criteria must be clearly met:

a. You must certify at the time that the existing study is submitted that the raw data and specimens from the study are available for audit and review and you must identify where they are available. This must be done in accordance with the requirements of the Good Laboratory Practice (GLP) regulation, 40 CFR Part 160. As stated in 40 CFR 160.3(j) " 'raw data' means any laboratory worksheets, records, memoranda, notes, or exact copies thereof, that are the result of original observations and activities of a study and are necessary for the reconstruction and evaluation of the report of that study. In the event that exact transcripts of raw data have been prepared (e.g., tapes which have been transcribed verbatim, dated, and verified accurate by signature), the exact copy or exact transcript may be substituted for the original source as raw data. 'Raw data' may include photographs, microfilm or microfiche copies, computer printouts, magnetic

media, including dictated observations, and recorded data from automated instruments." The term "specimens", according to 40 CFR 160.3(k), means "any material derived from a test system for examination or analysis."

b. Health and safety studies completed after May 1984 must also contain all GLP-required quality assurance and quality control information, pursuant to the requirements of 40 CFR Part 160. Registrants must also certify at the time of submitting the existing study that such GLP information is available for post-May 1984 studies by including an appropriate statement on or attached to the study signed by an authorized official or representative of the registrant.

c. You must certify that each study fulfills the acceptance criteria for the Guideline relevant to the study provided in the FIFRA Accelerated Reregistration Phase 3 Technical Guidance and that the study has been conducted according to the Pesticide Assessment Guidelines (PAG) or meets the purpose of the PAG (both available from NTIS). A study not conducted according to the PAG may be submitted to the Agency for consideration if the registrant believes that the study clearly meets the purpose of the PAG. The registrant is referred to 40 CFR 158.70 which states the Agency's policy regarding acceptable protocols. If you wish to submit the study, you must, in addition to certifying that the purposes of the PAG are met by the study, clearly articulate the rationale why you believe the study meets the purpose of the PAG, including copies of any supporting information or data. It has been the Agency's experience that studies completed prior to January 1970 rarely satisfied the purpose of the PAG and that necessary raw data are usually not available for such studies.

If you submit an existing study, you must certify that the study meets all requirements of the criteria outlined above.

If you know of a study pertaining to any requirement in this Notice which does not meet the criteria outlined above but does contain factual information regarding unreasonable adverse effects, you must notify the Agency of such a study. If such study is in the Agency's files, you need only cite it along with the notification. If not in the Agency's files, you must submit a summary and copies as required by PR Notice 86-5.

Option 5, Upgrading a Study -- If a study has been classified as partially acceptable and upgradeable, you may submit data to upgrade that study. The Agency will review the data submitted and determine if the requirement is satisfied. If the Agency decides the requirement is not satisfied, you may still be required to submit new data normally without any time extension. Deficient, but upgradeable studies will normally be classified as supplemental. However, it is important to note that not all studies classified as supplemental are upgradeable. If you have questions regarding the classification of a study or whether a study may be upgraded, call or write the contact person listed in Attachment A. If you submit data to

upgrade an existing study you must satisfy or supply information to correct all deficiencies in the study identified by EPA. You must provide a clearly articulated rationale of how the deficiencies have been remedied or corrected and why the study should be rated as acceptable to EPA. Your submission must also specify the MRID number(s) of the study which you are attempting to upgrade and must be in conformance with PR Notice 86-5.

Do not submit additional data for the purpose of upgrading a study classified as unacceptable and determined by the Agency as not capable of being upgraded.

This option should also be used to cite data that has been previously submitted to upgrade a study, but has not yet been reviewed by the Agency. You must provide the MRID number of the data submission as well as the MRID number of the study being upgraded.

The criteria for submitting an existing study, as specified in Option 4 above, apply to all data submissions intended to upgrade studies. Additionally your submission of data intended to upgrade studies must be accompanied by a certification that you comply with each of those criteria as well as a certification regarding protocol compliance with Agency requirements.

Option 6, Citing Existing Studies -- If you choose to cite a study that has been previously submitted to EPA, that study must have been previously classified by EPA as acceptable or it must be a study which has not yet been reviewed by the Agency. Acceptable toxicology studies generally will have been classified as "core-guideline" or "core minimum." For all other disciplines the classification would be "acceptable." With respect to any studies for which you wish to select this option you must provide the MRID number of the study you are citing and, if the study has been reviewed by the Agency, you must provide the Agency's classification of the study.

If you are citing a study of which you are not the original data submitter, you must submit a completed copy of EPA Form 8570-31, Certification with Respect to Data Compensation Requirements.

Registrants who select one of the above 6 options must meet all of the requirements described in the instructions for completing the Data Call-In Response Form and the Requirements Status and Registrant's Response Form, as appropriate.

III-D REQUESTS FOR DATA WAIVERS

If you request a waiver for product specific data because you believe it is inappropriate, you must attach a complete justification for the request, including technical reasons, data and references to relevant EPA regulations, guidelines or policies. (Note: any supplemental data must be submitted in the format required by PR Notice 86-5). This will be the only opportunity to state the reasons or provide information in support of your request. If the Agency approves your waiver request, you will not be required to supply the data pursuant to section 3(c)(2)(B) of FIFRA. If the Agency denies your waiver request, you must choose an option for meeting the data requirements of this Notice within 30 days of the receipt of the Agency's decision. ~~You must~~ indicate and submit the option chosen on the Requirements Status and Registrant's Response Form. Product specific data requirements for product chemistry, acute toxicity and efficacy (where appropriate) are required for all products and the Agency would grant a waiver only under extraordinary circumstances. You should also be aware that submitting a waiver request will not automatically extend the due date for the study in question.

Waiver requests submitted without adequate supporting rationale will be denied and the original due date will remain in force.

IV. CONSEQUENCES OF FAILURE TO COMPLY WITH THIS NOTICE

IV-A NOTICE OF INTENT TO SUSPEND

The Agency may issue a Notice of Intent to Suspend products subject to this Notice due to failure by a registrant to comply with the requirements of this Data Call-In Notice, pursuant to FIFRA section 3(c)(2)(B). Events which may be the basis for issuance of a Notice of Intent to Suspend include, but are not limited to, the following:

1. Failure to respond as required by this Notice within 90 days of your receipt of this Notice.

2. Failure to submit on the required schedule an acceptable proposed or final protocol when such is required to be submitted to the Agency for review.

3. Failure to submit on the required schedule an adequate progress report on a study as required by this Notice.

4. Failure to submit on the required schedule acceptable data as required by this Notice.

5. Failure to take a required action or submit adequate information pertaining to any option chosen to address the data requirements (e.g., any required action or information pertaining to submission or citation of existing studies or offers, arrangements, or arbitration on the sharing of costs or the formation of Task Forces, failure to comply with the terms of an agreement or arbitration concerning joint data development or failure to comply with any terms of a data waiver).

6. Failure to submit supportable certifications as to the conditions of submitted studies, as required by Section III-C of this Notice.

7. Withdrawal of an offer to share in the cost of developing required data.

8. Failure of the registrant to whom you have tendered an offer to share in the cost of developing data and provided proof of the registrant's receipt of such offer or failure of a registrant on whom you rely for a generic data exemption either to:

   a. inform EPA of intent to develop and submit the data required by this Notice on a Data Call-In Response Form and a Requirements Status and Registrant's Response Form;

   b. fulfill the commitment to develop and submit the data as required by this Notice; or

c. otherwise take appropriate steps to meet the requirements stated in this Notice, unless you commit to submit and do submit the required data in the specified time frame.

9. Failure to take any required or appropriate steps, not mentioned above, at any time following the issuance of this Notice.

IV-B. BASIS FOR DETERMINATION THAT SUBMITTED STUDY IS UNACCEPTABLE

The Agency may determine that a study (even if submitted within the required time) is unacceptable and constitutes a basis for issuance of a Notice of Intent to Suspend. The grounds for suspension include, but are not limited to, failure to meet any of the following:

1. EPA requirements specified in the Data Call-In Notice or other documents incorporated by reference (including, as applicable, EPA Pesticide Assessment Guidelines, Data Reporting Guidelines, and GeneTox Health Effects Test Guidelines) regarding the design, conduct, and reporting of required studies. Such requirements include, but are not limited to, those relating to test material, test procedures, selection of species, number of animals, sex and distribution of animals, dose and effect levels to be tested or attained, duration of test, and, as applicable, Good Laboratory Practices.

2. EPA requirements regarding the submission of protocols, including the incorporation of any changes required by the Agency following review.

3. EPA requirements regarding the reporting of data, including the manner of reporting, the completeness of results, and the adequacy of any required supporting (or raw) data, including, but not limited to, requirements referenced or included in this Notice or contained in PR 86-5. All studies must be submitted in the form of a final report; a preliminary report will not be considered to fulfill the submission requirement.

IV-C EXISTING STOCKS OF SUSPENDED OR CANCELLED PRODUCTS

EPA has statutory authority to permit continued sale, distribution and use of existing stocks of a pesticide product which has been suspended or cancelled if doing so would be consistent with the purposes of the Act.

The Agency has determined that such disposition by registrants of existing stocks for a suspended registration when a section 3(c)(2)(B) data request is outstanding would generally not be consistent with the Act's purposes. Accordingly, the Agency anticipates granting registrants permission to sell, distribute, or use existing stocks of suspended product(s) only in exceptional circumstances. If you believe such disposition of existing stocks of your product(s) which may be suspended for failure to comply with this Notice should be permitted, you have the burden of clearly demonstrating to EPA that granting such permission would be consistent with the Act.

You must also explain why an "existing stocks" provision is necessary, including a statement of the quantity of existing stocks and your estimate of the time required for their sale, distribution, and use. Unless you meet this burden the Agency will not consider any request pertaining to the continued sale, distribution, or use of your existing stocks after suspension.

If you request a voluntary cancellation of your product(s) as a response to this Notice and your product is in full compliance with all Agency requirements, you will have, under most circumstances, one year from the date your 90 day response to this Notice is due, to sell, distribute, or use existing stocks. Normally, the Agency will allow persons other than the registrant such as independent distributors, retailers and end users to sell, distribute or use such existing stocks until the stocks are exhausted. Any sale, distribution or use of stocks of voluntarily cancelled products containing an active ingredient for which the Agency has particular risk concerns will be determined on case-by-case basis.

Requests for voluntary cancellation received after the 90 day response period required by this Notice will not result in the Agency granting any additional time to sell, distribute, or use existing stocks beyond a year from the date the 90 day response was due unless you demonstrate to the Agency that you are in full compliance with all Agency requirements, including the requirements of this Notice. For example, if you decide to voluntarily cancel your registration six months before a 3 year study is scheduled to be submitted, all progress reports and other information necessary to establish that you have been conducting the study in an acceptable and good faith manner must have been submitted to the Agency, before EPA will consider granting an existing stocks provision.

SECTION V. REGISTRANTS' OBLIGATION TO REPORT POSSIBLE UNREASONABLE ADVERSE EFFECTS

Registrants are reminded that FIFRA section 6(a)(2) states that if at any time after a pesticide is registered a registrant has additional factual information regarding unreasonable adverse effects on the environment by the pesticide, the registrant shall submit the information to the Agency. Registrants must notify the Agency of any factual information they have, from whatever source, including but not limited to interim or preliminary results of studies, regarding unreasonable adverse effects on man or the environment. This requirement continues as long as the products are registered by the Agency.

SECTION VI. INQUIRIES AND RESPONSES TO THIS NOTICE

If you have any questions regarding the requirements and procedures established by this Notice, call the contact person(s) listed in Attachment A, the Data Call-In Chemical Status Sheet.

All responses to this Notice (other than voluntary cancellation requests and generic data exemption claims) must include a completed Data Call-In Response Form and a completed Requirements Status and Registrant's Response Form (Attachment B for generic data and Attachment C for product specific data) and any other documents required by this Notice, and should ~~be submitted to the contact~~ person(s) identified in Attachment A. If the voluntary cancellation or generic data exemption option is chosen, only the Data Call-In Response Form need be submitted.

The Office of Compliance Monitoring (OCM) of the Office of Pesticides and Toxic Substances (OPTS), EPA, will be monitoring the data being generated in response to this Notice.

Sincerely yours,

Peter Caulkins

Peter P. Caulkins Ph.D., Acting Director
Special Review and
Reregistration Division

Attachments

A- Data Call-In Chemical Status Sheet
B- Product-Specific Data Call-In Response Form
C- Requirements Status and Registrant's Response Form for the Product Specific Data Call-In
D- EPA Grouping of End-Use Products for Meeting Acute Toxicology Data Requirements for Reregistration
E- EPA Acceptance Criteria
F- List of Registrants Receiving This Notice
G- Cost Share and Data Compensation Forms, and Product Specific Data Report Form

Attachment A

Chemical Status Sheet

CEDARWOOD OIL DATA CALL[-]IN CHEMICAL STATUS SHEET

INTRODUCTION

You have been sent this Product Specific Data Call-In Notice because you have product(s) containing cedarwood oil.

This Product Specific Data Call-In Chemical Status Sheet, contains an overview of data required by this notice, and point of contact for inquiries pertaining to the reregistration of cedarwood oil. This attachment is to be used in conjunction with (1) the Product Specific Data Call-In Notice, (2) the Product Specific Data Call-In Response Form (Attachment B), (3) the Requirements Status and Registrant's Form (Attachment C), (4) EPA's Grouping of End[-]Use Products for Meeting Acute Toxicology Data Requirement (Attachment D), (5) the EPA Acceptance Criteria (Attachment E), (6) a list of registrants receiving this DCI (Attachment F) and (7) the Cost Share and Data Compensation Forms in replying to this cedarwood oil Product Specific Data Call[-]In (Attachment G). Instructions and guidance accompany each form.

DATA REQUIRED BY THIS NOTICE

The additional data requirements needed to complete the database for cedarwood oil are contained in the Requirements Status and Registrant's Response, Attachment C. The Agency has concluded that additional data on cedarwood oil are needed for specific products. These data are required to be submitted to the Agency within the time frame listed. These data are needed to fully complete the reregistration of all eligible cedarwood oil products.

INQUIRIES AND RESPONSES TO THIS NOTICE

If you have any questions regarding the generic database of cedarwood oil, please contact Ron Kendall at (703) 308-8068.

If you have any questions regarding the product specific data requirements and procedures established by this Notice, please contact Frank Rubis (703) 308-8184. All responses to this Notice for the Product Specific data requirements should be submitted to:

Frank Rubis, Product Manager, Team 81
Product Reregistration Branch
Special Review and Reregistration Division 7508W
~~Office of Pesticide~~ Programs
U.S. Environmental Protection Agency
Washington, D.C. 20460
RE: **Cedarwood Oil**

INSTRUCTIONS FOR COMPLETING THE DATA CALL-IN RESPONSE FORM FOR PRODUCT SPECIFIC DATA

Item 1-4. Already completed by EPA.

Item 5. If you wish to voluntarily cancel your product, answer "yes." If you choose this option, you will not have to provide the data required by the Data Call-In Notice and you will not have to complete any other forms. Further sale and distribution of your product after the effective date of cancellation must be in accordance with the Existing Stocks provision of the Data Call-In Notice (Section IV-C).

Item 6. Not applicable since this form calls in product specific data only. However, if your product is identical to another product and you qualify for a data exemption, you must respond with "yes" to Item 7a (MUP) or 7B (EUP) on this form, provide the EPA registration numbers of your source(s); you would not complete the "Requirements Status and Registrant's Response" form. Examples of such products include repackaged products and Special Local Needs (Section 24c) products which are identical to federally registered products.

Item 7a. For each manufacturing use product (MUP) for which you wish to maintain registration, you must agree to satisfy the data requirements by responding "yes."

Item 7b. For each end use product (EUP) for which you wish to maintain registration, you must agree to satisfy the data requirements by responding "yes." If you are requesting a data waiver, answer "yes" here; in addition, on the "Requirements Status and Registrant's Response" form under Item 9, you must respond with Option 7 (Waiver Request) for each study for which you are requesting a waiver. See Item 6 with regard to identical products and data exemptions.

Items 8-11. Self-explanatory.

NOTE: You may provide additional information that does not fit on this form in a signed letter that accompanies this form. For example, you may wish to report that your product has already been transferred to another company or that you have already voluntarily canceled this product. For these cases, please supply all relevant details so that EPA can ensure that its records are correct.

INSTRUCTIONS FOR COMPLETING THE REQUIREMENTS STATUS AND REGISTRANT'S RESPONSE FORM FOR PRODUCT SPECIFIC DATA

Item 1-3 Completed by EPA. Note the unique identifier number assigned by EPA in Item 3. This number must be used in the transmittal document for any data submissions in response to this Data Call-In Notice.

Item 4. The guideline reference numbers of studies required to support the product's continued registration are identified. These guidelines, in addition to the requirements specified in the Notice, govern the conduct of the required studies. Note that series 61 and 62 in product chemistry are now listed under 40 CFR 158.155 through 158.180, Subpart C.

Item 5. The study title associated with the guideline reference number is identified.

Item 6. The use pattern(s) of the pesticide associated with the product specific requirements is (are) identified. For most product specific data requirements, all use patterns are covered by the data requirements. In the case of efficacy data, the required studies only pertain to products which have the use sites and/or pests indicated.

Item 7. The substance to be tested is identified by EPA. For product specific data, the product as formulated for sale and distribution is the test substance, except in rare cases.

Item 8. The due date for submission of each study is identified. It is normally based on 8 months after issuance of the Reregistration Eligibility Document unless EPA determines that a longer time period is necessary.

Item 9. Enter only one of the following response codes for each data requirement to show how you intend to comply with the data requirements listed in this table. Fuller descriptions of each option are contained in the Data Call-In Notice.

1. I will generate and submit data by the specified due date (Developing Data). By indicating that I have chosen this option, I certify that I will comply with all the requirements pertaining to the conditions for submittal of this study as outlined in the Data Call-In Notice. By the specified due date, I will also submit: (1) a completed "Certification With Respect To Data Compensation Requirements" form (EPA Form 8570-29) and (2) two completed and signed copies of the Confidential Statement of Formula (EPA Form 8570-4).

2. I have entered into an agreement with one or more registrants to develop data jointly (Cost Sharing). I am submitting a copy of this agreement. I understand that this option is available only for acute toxicity or certain efficacy data and only if EPA indicates in an attachment to this Notice that my product is similar

enough to another product to qualify for this option. I certify that another party in the agreement is committing to submit or provide the required data; if the required study is not submitted on time, my product may be subject to suspension. By the specified due date, I will also submit: (1) a completed "Certification With Respect To Data Compensation Requirements" form (EPA Form 8570-29) and (2) two completed and signed copies of the Confidential Statement of Formula (EPA Form 8570-4).

3. I have made offers to share in the cost to develop data (Offers to Cost Share). I understand that this option is available only for acute toxicity or certain efficacy data and only if EPA indicates in an attachment to this Data Call-In Notice that my product is similar enough to another product to qualify for this option. I am submitting evidence that I have made an offer to another registrant (who has an obligation to submit data) to share in the cost of that data. I am also submitting a completed "Certification of Offer to Cost Share in the Development Data" form. I am including a copy of my offer and proof of the other registrant's receipt of that offer. I am identifying the party which is committing to submit or provide the required data; if the required study is not submitted on time, my product may be subject to suspension. I understand that other terms under Option 3 in the Data Call-In Notice (Section III-C.1.) apply as well. By the specified due date, I will also submit: (1) a completed "Certification With Respect To Data Compensation Requirements" form (EPA Form 8570-29) and (2) two completed and signed copies of the Confidential Statement of Formula (EPA Form 8570-4).

4. By the specified due date, I will submit an existing study that has not been submitted previously to the Agency by anyone (Submitting an Existing Study). I certify that this study will meet all the requirements for submittal of existing data outlined in Option 4 in the Data Call-In Notice (Section III-C.1.) and will meet the attached acceptance criteria (for acute toxicity and product chemistry data). I will attach the needed supporting information along with this response. I also certify that I have determined that this study will fill the data requirement for which I have indicated this choice. By the specified due date, I will also submit a completed "Certification With Respect To Data Compensation Requirements" form (EPA Form 8570-29) to show what data compensation option I have chosen. By the specified due date, I will also submit: (1) a completed "Certification With Respect To Data Compensation Requirements" form (EPA Form 8570-29) and (2) two completed and signed copies of the Confidential Statement of Formula (EPA Form 8570-4).

5. By the specified due date, I will submit or cite data to upgrade a study classified by the Agency as partially acceptable and upgradable (Upgrading a Study). I will submit evidence of the Agency's review indicating that the study may be upgraded and what information is required to do so. I will provide the MRID or Accession number of the study at the due date. I understand that the conditions for this option outlined Option 5 in the Data Call-In Notice (Section III-C.1.) apply. By the specified due date, I will also submit: (1) a completed

"Certification With Respect To Data Compensation Requirements" form (EPA Form 8570-29) and (2) two completed and signed copies of the Confidential Statement of Formula (EPA Form 8570-4).

6. By the specified due date, I will cite an existing study that the Agency has classified as acceptable or an existing study that has been submitted but not reviewed by the Agency (Citing an Existing Study). If I am citing another registrant's study, I understand that this option is available only for acute toxicity or certain efficacy data and only if the cited study was conducted on my product, an identical product or a product which EPA has "grouped" with one or more other products for purposes of depending on the same data. I may also choose this option if I am citing my own data. In either case, I will provide the MRID or Accession number(s) for the cited data on a "Product Specific Data Report" form or in a similar format. By the specified due date, I will also submit: (1) a completed "Certification With Respect To Data Compensation Requirements" form (EPA Form 8570-29) and (2) two completed and signed copies of the Confidential Statement of Formula (EPA Form 8570-4).

7. I request a waiver for this study because it is inappropriate for my product (Waiver Request). I am attaching a complete justification for this request, including technical reasons, data and references to relevant EPA regulations, guidelines or policies. [Note: any supplemental data must be submitted in the format required by P.R. Notice 86-5]. I understand that this is my only opportunity to state the reasons or provide information in support of my request. If the Agency approves my waiver request, I will not be required to supply the data pursuant to Section 3(c)(2)(B) of FIFRA. If the Agency denies my waiver request, I must choose a method of meeting the data requirements of this Notice by the due date stated by this Notice. In this case, I must, within 30 days of my receipt of the Agency's written decision, submit a revised "Requirements Status and Registrant's Response" Form indicating the option chosen. I also understand that the deadline for submission of data as specified by the original data call-in notice will not change. By the specified due date, I will also submit: (1) a completed "Certification With Respect To Data Compensation Requirements" form (EPA Form 8570-29) and (2) two completed and signed copies of the Confidential Statement of Formula (EPA Form 8570-4).

Items 10-13. Self-explanatory.

NOTE: You may provide additional information that does not fit on this form in a signed letter that accompanies this form. For example, you may wish to report that your product has already been transferred to another company or that you have already voluntarily canceled this product. For these cases, please supply all relevant details so that EPA can ensure that its records are correct.

United States Environmental Protection Agency
Washington, D. C. 20460

DATA CALL-IN RESPONSE

Form Approved

OMB No. 2070-0107
2070-0057

Approval Expires 03-31-96

INSTRUCTIONS: Please type or print in ink. Please read carefully the attached instructions and supply the information requested on this form. Use additional sheet(s) if necessary.

1. Company name and Address	2. Case # and Name	3. Date and Type of DCI
SAMPLE COMPANY NO STREET ADDRESS NO CITY, XX 00000	3150 Wood oils and gums	PRODUCT SPECIFIC

4. EPA Product Registration	5. I wish to cancel this product registration voluntarily.	6. Generic Data		7. Product Specific Data	
		6a. I am claiming a Generic Data Exemption because I obtain the active ingredient from the source EPA registration number listed below.	6b. I agree to satisfy Generic Data requirements as indicated on the attached form entitled "Requirements Status and Registrant's Response."	7a. My product is a MUP and I agree to satisfy the MUP requirements on the attached form entitled "Requirements Status and Registrant's Response."	7b. My product is an EUP and I agree to satisfy the EUP requirements on the attached form entitled "Requirements Status and Registrant's Response."
NNNNNN-NNNNN		N.A.	N.A.		

8. Certification

I certify that the statements made on this form and all attachments are true, accurate, and complete. I acknowledge that any knowingly false or misleading statement may be punishable by fine, imprisonment or both under applicable law.

Signature and Title of Company's Authorized Representative_____

9. Date

10. Name of Company Contact

11. Phone Number

United States Environmental Protection Agency
Washington, D. C. 20460

REQUIREMENTS STATUS AND REGISTRANT'S RESPONSE

Form Approved
OMB No. 2070-0107
2070-0057
Approval Expires 03-31-96

INSTRUCTIONS: Please type or print in ink. Please read carefully the attached instructions and supply the information requested on this form. Use additional sheet(s) if necessary.

1. Company name and Address	2. Case # and Name	3. Date and Type of DCI
SAMPLE COMPANY NO STREET ADDRESS NO CITY, XX 00000	3150 Wood oils and gums EPA Reg. No. NNNNNN-NNNNN	PRODUCT SPECIFIC ID# NNNNNN-RD-NNNN

4. Guideline Requirement Number	5. Study Title	PROTOCOL	Progress Reports 1	2	3	6. Use Pattern	7. Test Substance	8. Time Frame	9. Registrant Response
	Prod Chem - Regular Chemical								
61-1	Product identity & composition (1)					ABCDEFGHIJKLMNO	MP/EP	8 mos.	
61-2(a)	Descriptn starting materials, (1,2) producton & formulatn process					ABCDEFGHIJKLMNO	MP/EP and TGAI	8 mos.	
61-2(b)	Discussion of formation of (1,3) impurities					ABCDEFGHIJKLMNO	MP/EP and TGAI	8 mos.	
62-1	Preliminary analysis (1,4)					ABCDEFGHIJKLMNO	MP/EP and TGAI	8 mos.	
62-2	Certification of limits (1,5)					ABCDEFGHIJKLMNO	MP/EP	8 mos.	
62-3	Analytical method (1)					ABCDEFGHIJKLMNO	MP/EP	8 mos.	
63-2	Color					ABCDEFGHIJKLMNO	MP/EP and TGAI	8 mos.	
63-3	Physical state					ABCDEFGHIJKLMNO	MP/EP and TGAI	8 mos.	
63-4	Odor					ABCDEFGHIJKLMNO	MP/EP and TGAI	8 mos.	
63-5	Melting point (6)					ABCDEFGHIJKLMNO	TGAI	8 mos.	
63-6	Boiling point (7)					ABCDEFGHIJKLMNO	TGAI	8 mos.	
63-7	Density					ABCDEFGHIJKLMNO	MP/EP and TGAI	8 mos.	

10. Certification

I certify that the statements made on this form and all attachments are true, accurate, and complete. I acknowledge that any knowingly false or misleading statement may be punishable by fine, imprisonment or both under applicable law.

Signature and Title of Company's Authorized Representative_____

11. Date

12. Name of Company Contact

13. Phone Number

United States Environmental Protection Agency
Washington, D. C. 20460

REQUIREMENTS STATUS AND REGISTRANT'S RESPONSE

Form Approved
OMB No. 2070-0107
2070-0057
Approval Expires 03-31-96

INSTRUCTIONS: Please type or print in ink. Please read carefully the attached instructions and supply the information requested on this form. Use additional sheet(s) if necessary.

1. Company name and Address	2. Case # and Name	3. Date and Type of DCI
SAMPLE COMPANY NO STREET ADDRESS NO CITY, XX 00000	3150 Wood oils and gums EPA Reg. No. NNNNNN-NNNNN	PRODUCT SPECIFIC ID# NNNNNN-RD-NNNN

4. Guideline Requirement Number	5. Study Title		PROTOCOL	Progress Reports 1	2	3	6. Use Pattern	7. Test Substance	8. Time Frame	9. Registrant Response
63-8	Solubility						ABCDEFGHIJKLMNO	TGAI/PAI	8 mos.	
63-9	Vapor pressure						ABCDEFGHIJKLMNO	TGAI/PAI	8 mos.	
63-10	Dissociation constant						ABCDEFGHIJKLMNO	TGAI/PAI	8 mos.	
63-11	Octanol/water partition coefficient	(8)					ABCDEFGHIJKLMNO	PAI	8 mos.	
63-12	pH	(9)					ABCDEFGHIJKLMNO	MP/EP and TGAI	8 mos.	
63-13	Stability						ABCDEFGHIJKLMNO	MP/EP	8 mos.	
63-14	Oxidizing or reducing action	(10)					ABCDEFGHIJKLMNO	MP/EP	8 mos.	
63-15	Flammability	(11)					ABCDEFGHIJKLMNO	MP/EP	8 mos.	
63-16	Explodability	(12)					ABCDEFGHIJKLMNO	MP/EP	8 mos.	
63-17	Storage stability						ABCDEFGHIJKLMNO	MP/EP	8 mos.	
63-18	Viscosity	(13)					ABCDEFGHIJKLMNO	MP/EP	8 mos.	
63-19	Miscibility	(14)					ABCDEFGHIJKLMNO	MP/EP	8 mos.	
63-20	Corrosion characteristics						ABCDEFGHIJKLMNO	MP/EP	8 mos.	
63-21	Dielectric breakdown voltage	(15)					ABCDEFGHIJKLMNO	MP/EP	8 mos.	
	Acute Toxic - Regular Chemical									
81-1	Acute oral toxicity-rat	(1,36,37)					ABCDEFGHIJKLMNO	MP/EP and TGAI	8 mos.	
81-2	Acute dermal toxicity-rabbit/rat	(1,2,37)					ABCDEFGHIJKLMNO	MP/EP and TGAI	8 mos.	
81-3	Acute inhalation toxicity-rat	(3)					ABCDEFGHIJKLMNO	MP/EP and TGAI	8 mos.	

Initial to indicate certification as to information on this page (full text of certification is on page one).

Date

United States Environmental Protection Agency
Washington, D. C. 20460

REQUIREMENTS STATUS AND REGISTRANT'S RESPONSE

Form Approved
OMB No. 2070-0107
2070-0057
Approval Expires 03-31-96

INSTRUCTIONS: Please type or print in ink. Please read carefully the attached instructions and supply the information requested on this form. Use additional sheet(s) if necessary.

1. Company name and Address	2. Case # and Name	3. Date and Type of DCI
SAMPLE COMPANY NO STREET ADDRESS NO CITY, XX 00000	3150 Wood oils and gums EPA Reg. No. NNNNNN-NNNNN	PRODUCT SPECIFIC ID# NNNNNN-RD-NNNN

4. Guideline Requirement Number	5. Study Title	PROTOCOL	Progress Reports 1	2	3	6. Use Pattern	7. Test Substance	8. Time Frame	9. Registrant Response
81-4	Primary eye irritation-rabbit (2)					ABCDEFGHIJKLMNO	MP/EP	8 mos.	
81-5	Primary dermal irritation (1,2)					ABCDEFGHIJKLMNO	MP/EP	8 mos.	
81-6	Dermal sensitization (4)					ABCDEFGHIJKLMNO	MP/EP	8 mos.	
	Efficacy - Invertebrate Control Agents								
	Premises Treatments								
95-11	Laboratory efficacy evaluation (1,3,4)					KLM O	EP	8 mos.	

Initial to indicate certification as to information on this page (full text of certification is on page one).

Date

United States Environmental Protection Agency
Washington, D. C. 20460

FOOTNOTES AND KEY DEFINATIONS FOR GUIDELINE REQUIREMENTS

Case # and Name: 3150 Wood oils and gums

...ct; EP = end-use product; provided formulators purchase their active ingredient(s) from a registered source, they need not submit or cite data pertaining to the purchased product. [NOTE: If a product is a 100 percent repackage of another registered product that is purchased, and any use for the product does not differ from those of the purchased and registered source, users are not subject to any data requirements identified in the tables.]; TEP = typical end-use product: TGAI = technical grade of the active ingredient; PAI = "pure" active ingredient; PAIRA = "pure" active ingredient, radiolabeled.

Use Categories Key:

A - Terrestrial food crop	B - Terrestrial food feed crop	C - Terrestrial nonfood crop	D - Aquatic food crop	E - Aquatic nonfood outdoor
F - Aquatic nonfood industrial	G - Aquatic nonfood residential	H - Greenhouse food crop	I - Greenhouse nonfood crop	J - Forestry
K - Residential outdoor	L - Indoor food	M - Indoor nonfood	N - Indoor Medical	O - Indoor residential

Footnotes: [The following notes are referenced in column two (5. Study Title) of the REQUIREMENTS STATUS AND REGISTRANT'S RESPONSE form.]

Prod Chem - Regular Chemical

1 Requirements pertaining to product identity, composition, analysis, and certification of ingredients are detailed further in the following sections: *158.155 for product identity and composition (61-1); *158.160, 158.162, and 158.165 for description of starting materials and manufacturing process (61-2); *158.167 for discussion of formation of impurities (61-3); *158.170 for preliminary analysis (62-1); *158.175 for certification of limits (62-2); and *158.180 for enforcement analytical methods (62-3).
2 A schematic diagram and/or brief description of the production process will suffice if the pesticide is not already under full scale production and an experimental use permit is being sought.
3 If the pesticide is not already under full scale production and an experimental use permit is sought, a discussion of unintentional ingredients shall be submitted to the extent this information is available.
4 To support registration of an MP or EP, whether produced by an integrated system or not, the technical grade of Active Ingredient must be analyzed. If the technical grade of Active Ingredient cannot be isolated, a statement of composition of the practical equivalent of the technical grade of Active Ingredient must be submitted. Data on EPs or MPs will be required on a case-by-case basis.
5 Certified limits are not required for inert ingredients in products proposed for experimental use.
6 Required if technical chemical is solid at room temperature.
7 Required if technical chemical is liquid at room temperature.
8 Required if technical chemical is organic and non-polar.
9 Required if test substances are dispersible with water.
10 Required if product contains an oxidizing or reducing agent.
11 Required if product contains combustible liquids.
12 Required if product is potentially explosive.
13 Required if product is a liquid.
14 Required if product is an emulsifiable liquid and is to be diluted with petroleum solvents.
15 Required if end-use product is liquid and is to be used around electrical equipment.

Acute Toxic - Regular Chemical

1 Not required if test material is a gas or highly volatile.
2 Not required if test material is corrosive to skin or has pH less than 2 or greater than 11.5; such a product will be classified as Toxicity Category I on the basis of potential eye and dermal irritation effects.

United States Environmental Protection Agency
Washington, D. C. 20460

FOOTNOTES AND KEY DEFINATIONS FOR GUIDELINE REQUIREMENTS

Case # and Name: 3150 Wood oils and gums

Footnotes (cont.):

3 Required if the product consists of, or under conditions of use will result in, an inhalable material (e. g., gas, volatile substances, or aerosol/particulate).
4 required unless repeated dermal exposure does not occur under conditions of use.
... (acute, subchronic, and/or chronic) is required for organophospates, and may be required for other cholinesterase inhibitors and other pesticides ... ed a potential to adverse affect the visual system. Registrants should consult with the agency for development of protocols and methodology ... udies.
... of the EP dilution is required if it can be reasonably anticipated that the results of such testing may meet the criteria for restriction to use by certified applicators specified in 40 CFR 152.170(b) or the criteria for initiation of special review specified in 40 CFR 154.7 (a)(1).

Efficacy - Invertebrate Control Agents

1 The agency has waived all requirements to submit efficacy data for invertebrate control agents for nonpublic health uses. However, each registrant must ensure through testing that his products are efficacious when used in accordance with label directions and commenly accepted pest control practices. The registrant must develop and maintain the relevant data upon which the determination of efficacy is based. The Agency reserves the right to require, on a case-by-case basis (e.g., significant new uses or benefits data in cases of special reviews) submission of efficacy data for any pesticide product, registered or proposed for registration when necessary.
3 Efficacy evaluations can be conducted under laboratory, greenhouse, or field conditions.
4 Required to be developed and maintained in the Reqgistrant's file for all pests claimed on the label when resistance to the pestcide has been demonstrated.

Attachment D

EPA Grouping of End-Use Products for Meeting Data Requirements for Reregistration

EPA'S DECISION ON BATCHING PRODUCTS CONTAINING CEDAR WOOD OIL FOR PURPOSES OF MEETING ACUTE TOXICITY DATA REQUIREMENTS FOR REREGISTRATION

In an effort to reduce the time, resources and number of animals needed to fulfill the acute toxicity data requirements for reregistration of products containing the active ingredient cedar wood oil, the Agency considered batching products. This process involves grouping similar products for purposes of acute toxicity. Factors considered in the sorting process include each product's active and inert ingredients (identity, percent composition and biological activity), type of formulation (e.g., emulsifiable concentrate, aerosol, wettable powder, granular, etc.), and labeling (e.g., signal word, use classification, precautionary labeling, etc.). Note that the Agency is not describing batched products as "substantially similar" since some products within a batch may not be considered chemically similar or have identical use patterns.

Batching has been accomplished using the information described above as available. Acute toxicity data on individual products has frequently been found to be incomplete. Notwithstanding the batching process, the Agency reserves the right to require, at any time, acute toxicity data for an individual product should the need arise.

Registrants of products within a batch may choose to cooperatively generate, submit or cite a single battery of six acute toxicological studies to represent all the products within that batch. It is the registrants' option to participate in the process with all other registrants, only some of the other registrants, or only their own products within a batch, or to generate all the required acute toxicological studies for each of their own products. If a registrant chooses to rely upon previously submitted acute toxicity data, he/she may do so provided that the data base is complete and valid by today's standards (see acceptance criteria attached), the formulation tested is considered by EPA to be similar for acute toxicity, and the formulation has not been significantly altered since submission and acceptance of the acute toxicity data. Regardless of whether new data is generated or existing data is cited, the registrant must clearly identify the material tested by its EPA registration number.

In deciding how to meet the product specific data requirements, registrants must follow the directions given in the Data Call-In Notice and its attachments appended to the RED. The DCI Notice contains two response forms which are to be completed and submitted to the Agency within 90 days of receipt. The first form, "Data Call-In Response", asks whether the registrant will meet the data requirements for each product. The second form, "Requirements Status and Registrant's Response", lists the product specific data required for each product, including the standard six acute toxicity tests. A registrant who wishes to participate in a batch must decide whether he/she will provide the data or depend on someone else to do so. If a registrant supplies the data to support a batch of products, he/she must select one of the following options: Developing Data (Option 1), Submitting an Existing Study (Option 4), Upgrading an Existing Study (Option 5), or Citing an Existing Study (Option 6). If a registrant depends on another's data, he/she must choose among: Cost Sharing (Option 2), Offers to Cost Share (Option 3) or Citing an Existing Study (Option 6). If a registrant does not want to participate in a batch, the choices are Options 1, 4, 5 or 6. However, a registrant should know that choosing not to participate in a batch does not preclude other registrants in the batch form citing his/her studies and offering to cost share (Option 3) those studies.

Table I lists the products of Batch 1.

Table I.

Batch No.	EPA Reg. No.	% of Cedar Wood Oil	Formulation Type
1	65555-1	5.17	block
	65813-1	4.40	block
	66211-1	4.40	block

Table II lists the products which could not be batched. These products were not considered similar for purposes of acute toxicity.
The registrants of these products are responsible for meeting the acute toxicity data requirements specified in the data matrix for end-use products.

Table II.

EPA Reg. No.	% of Cedar Wood Oil & other Active Ingredients	Formulation Type
63380-1	0.48	aerosol
42443-1	0.50 Oil of Pennyroyal 2.00 Oil of Eucalyptus 1.00 Oil of Citronella 0.50 Oil of Rue 0.12	collar

ATTACHMENT E

EPA ACCEPTANCE CRITERIA

SUBDIVISION D

Guideline	Study Title
Series 61	Product Identity and Composition
Series 62	Analysis and Certification of Product Ingredients
Series 63	Physical and Chemical Characteristics

61 Product Identity and Composition

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1.____ Name of technical material tested (include product name and trade name, if appropriate).

2.____ Name, nominal concentration, and certified limits (upper and lower) for each active ingredient and each intentionally-added inert ingredient.

3.____ Name and upper certified limit for each impurity or each group of impurities present at $\geq$ 0.1% by weight and for certain toxicologically significant impurities (e.g., dioxins, nitrosamines) present at $<$0.1%.

4.____ Purpose of each active ingredient and each intentionally-added inert.

5.____ Chemical name from Chemical Abstracts index of Nomenclature and Chemical Abstracts Service (CAS) Registry Number for each active ingredient and, if available, for each intentionally-added inert.

6.____ Molecular, structural, and empirical formulas, molecular weight or weight range, and any company assigned experimental or internal code numbers for each active ingredient.

7.____ Description of each beginning material in the manufacturing process.
____ EPA Registration Number if registered; for other beginning materials, the following:
____ Name and address of manufacturer or supplier.
____ Brand name, trade name or commercial designation.
____ Technical specifications or data sheets by which manufacturer or supplier describes composition, properties or toxicity.

8.____Description of manufacturing process.
____ Statement of whether batch or continuous process.

____ Relative amounts of beginning materials and order in which they are added.
____ Description of equipment.
____ Description of physical conditions (temperature, pressure, humidity) controlled in each step and the parameters that are maintained.
____ Statement of whether process involves intended chemical reactions.
____ Flow chart with chemical equations for each intended chemical reaction.
____ Duration of each step of process.
____ Description of purification procedures.
____ Description of measures taken to assure quality of final product.

9.____ Discussion of formation of impurities based on established chemical theory addressing (1) each impurity which may be present at $\geq$ 0.1% or was found at $\geq$ 0.1% by product analyses and (2) certain toxicologically significant impurities (see #3).

62 Analysis and Certification of Product Ingredients

ACCEPTANCE CRITERIA

The following criteria apply to the technical grade of the active ingredient being reregistered. Use a table to present the information in items 6, 7, and 8.

Does your study meet the following acceptance criteria?

1.____ Five or more representative samples (batches in case of batch process) analyzed for each active ingredient and all impurities present at $\geq$ 0.1%.

2.____ Degree of accountability or closure $\geq$ ca 98%.

3.____ Analyses conducted for certain trace toxic impurities at lower than 0.1% (examples, nitrosamines in the case of products containing dinitroanilines or containing secondary or tertiary amines/alkanolamines plus nitrites; polyhalogenated dibenzodioxins and dibenzofurans). [Note that in the case of nitrosamines both fresh and stored samples must be analyzed.].

4.____ Complete and detailed description of each step in analytical method used to analyze above samples.

5.____ Statement of precision and accuracy of analytical method used to analyze above samples.

6.____ Identities and quantities (including mean and standard deviation) provided for each analyzed ingredient.

7.____ Upper and lower certified limits proposed for each active ingredient and intentionally added inert along with explanation of how the limits were determined.

8.____ Upper certified limit proposed for each impurity present at $\geq$ 0.1% and for certain toxicologically significant impurities at $<$0.1% along with explanation of how limit determined.

9.____ Analytical methods to verify certified limits of each active ingredient and impurities (latter not required if exempt from requirement of tolerance or if generally recognized as safe by FDA) are fully described.

10.____ Analytical methods (as discussed in #9) to verify certified limits validated as to their precision and accuracy.

63 Physical and Chemical Characteristics

ACCEPTANCE CRITERIA

The following criteria apply to the technical grade of the active ingredient being reregistered.

Does your study meet the following acceptance criteria?

63-2 Color

____ Verbal description of coloration (or lack of it)
____ Any intentional coloration also reported in terms of Munsell color system

63-3 Physical State

____ Verbal description of physical state provided using terms such as "solid, granular, volatile liquid"
____ Based on visual inspection at about 20-25° C

63-4 Odor

____ Verbal description of odor (or lack of it) using terms such as "garlic-like, characteristic of aromatic compounds"
____ Observed at room temperature

63-5 Melting Point

____ Reported in °C
____ Any observed decomposition reported

63-6 Boiling Point

____ Reported in °C
____ Pressure under which B.P. measured reported
____ Any observed decomposition reported

63-7 Density, Bulk Density, Specific Gravity

____ Measured at about 20-25° C
____ Density of technical grade active ingredient reported in g/ml or the specific gravity of liquids reported with reference to water at 20° C. [Note: Bulk density of registered products may be reported in lbs/ft^3 or lbs/gallon.]

63-8 Solubility

____ Determined in distilled water and representative polar and non-polar solvents, including those used in formulations and analytical methods for the pesticide
____ Measured at about 20-25° C
____ Reported in g/100 ml (other units like ppm acceptable if sparingly soluble)

63-9 Vapor Pressure

____ Measured at 25° C (or calculated by extrapolation from measurements made at higher temperature if pressure too low to measure at 25° C)
____ Experimental procedure described
____ Reported in mm Hg (torr) or other conventional units

63-10 Dissociation Constant

____ Experimental method described
____ Temperature of measurement specified (preferably about 20-25°C)

63-11 Octanol/water Partition Coefficient

____ Measured at about 20-25° C
____ Experimentally determined and description of procedure provided (preferred method-45 Fed. Register 77350)
____ Data supporting reported value provided

63-12 pH

____ Measured at about 20-25° C
____ Measured following dilution or dispersion in distilled water

63-13 Stability

____ Sensitivity to metal ions and metal determined
____ Stability at normal and elevated temperatures
____ Sensitivity to sunlight determined

SUBDIVISION F

Guideline	Study Title
81-1	Acute Oral Toxicity in the Rat
81-2	Acute Dermal Toxicity in the Rat, Rabbit or Guinea Pig
81-3	Acute Inhalation Toxicity in the Rat
81-4	Primary Eye Irritation in the Rabbit
81-5	Primary Dermal Irritation Study
81-6	Dermal Sensitization in the Guinea Pig

81-1 Acute Oral Toxicity in the Rat

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1.____ Identify material tested (technical, end-use product, etc).
2.____ At least 5 young adult rats/sex/group.
3.____ Dosing, single oral may be administered over 24 hrs.
4.*___ Vehicle control if other than water.
5.____ Doses tested, sufficient to determine a toxicity category or a limit dose (5000 mg/kg).
6.____ Individual observations at least once a day.
7.____ Observation period to last at least 14 days, or until all test animals appear normal whichever is longer.
8.____ Individual daily observations.
9.____ Individual body weights.
10.____ Gross necropsy on all animals.

Criteria marked with an * are supplemental and may not be required for every study.

81-2 Acute Dermal toxicity in the Rat, Rabbit or Guinea Pig

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1._____ Identify material tested (technical, end-use product, etc).
2._____ At least 5 animals/sex/group.
3.*____ Rats 200-300 gm, rabbits 2.0-3.0 kg or guinea pigs 350-450 gm.
4._____ Dosing, single dermal.
5._____ Dosing duration at least 24 hours.
6.*____ Vehicle control, only if toxicity of vehicle is unknown.
7._____ Doses tested, sufficient to determine a toxicity category or a limit dose (2000 mg/kg).
8._____ Application site clipped or shaved at least 24 hours before dosing.
9._____ Application site at least 10% of body surface area.
10._____ Application site covered with a porous nonirritating cover to retain test material and to prevent ingestion.
11._____ Individual observations at least once a day.
12._____ Observation period to last at least 14 days.
13._____ Individual body weights.
14._____ Gross necropsy on all animals.

Criteria marked with an * are supplemental and may not be required for every study.

81-3 Acute Inhalation Toxicity in the Rat

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1.____ Identify material tested (technical, end-use product, etc).
2.____ Product is a gas, a solid which may produce a significant vapor hazard based on toxicity and expected use or contains particles of inhalable size for man (aerodynamic diameter 15 μm or less).
3.____ At least 5 young adult rats/sex/group.
4.____ Dosing, at least 4 hours by inhalation.
5.____ Chamber air flow dynamic, at least 10 air changes/hour, at least 19% oxygen content.
6.____ Chamber temperature, 22° C ($\pm$2°), relative humidity 40-60%.
7.____ Monitor rate of air flow.
8.____ Monitor actual concentrations of test material in breathing zone.
9.____ Monitor aerodynamic particle size for aerosols.
10.___ Doses tested, sufficient to determine a toxicity category or a limit dose (5 mg/L actual concentration of respirable substance).
11.___ Individual observations at least once a day.
12.___ Observation period to last at least 14 days.
13.___ Individual body weights.
14.___ Gross necropsy on all animals.

81-4 Primary Eye Irritation in the Rabbit

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1.____ Identify material tested (technical, end-use product, etc).
2.____ Study not required if material is corrosive, causes severe dermal irritation or has a pH of ≤ 2 or ≥ 11.5.
3.____ 6 adult rabbits.
4.____ Dosing, instillation into the conjunctival sac of one eye per animal.
5.____ Dose, 0.1 ml if a liquid; 0.1 ml or not more than 100 mg if a solid, paste or particulate substance.
6.____ Solid or granular test material ground to a fine dust.
7.____ Eyes not washed for at least 24 hours.
8.____ Eyes examined and graded for irritation before dosing and at 1, 24, 48 and 72 hr, then daily until eyes are normal or 21 days (whichever is shorter).
9.*___ Individual daily observations.

Criteria marked with an * are supplemental and may not be required for every study.

81-5 Primary Dermal Irritation Study

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1.____ Identify material tested (technical, end-use product, etc).
2.____ Study not required if material is corrosive or has a pH of ≤ 2 or ≥ 11.5.
3.____ 6 adult animals.
4.____ Dosing, single dermal.
5.____ Dosing duration 4 hours.
6.____ Application site shaved or clipped at least 24 hours prior to dosing.
7.____ Application site approximately 6 cm^2.
8.____ Application site covered with a gauze patch held in place with nonirritating tape.
9.____ Material removed, washed with water, without trauma to application site.
10.____ Application site examined and graded for irritation at 1, 24, 48 and 72 hr, then daily until normal or 14 days (whichever is shorter).
11.*___ Individual daily observations.

Criteria marked with an * are supplemental and may not be required for every study.

81-6 Dermal Sensitization in the Guinea Pig

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1.___ Identify material tested (technical, end-use product, etc).
2.___ Study not required if material is corrosive or has a pH of ≤ 2 or ≥ 11.5.
3.___ One of the following methods is utilized:
   _____ Freund's complete adjuvant test
   _____ Guinea pig maximization test
   _____ Split adjuvant technique
   _____ Buehler test
   _____ Open epicutaneous test
   _____ Mauer optimization test
   _____ Footpad technique in guinea pig.
4.___ Complete description of test.
5.*__ Reference for test.
6.___ Test followed essentially as described in reference document.
7.___ Positive control included (may provide historical data conducted within the last 6 months).

Criteria marked with an * are supplemental and may not be required for every study.

ATTACHMENT F

LIST OF ALL REGISTRANTS SENT THIS DATA CALL-IN NOTICE

ATTACHMENT G

COST SHARE AND DATA COMPENSATION FORMS

United States Environmental Protection Agency
Washington, DC 20460

CERTIFICATION OF OFFER TO COST SHARE IN THE DEVELOPMENT OF DATA

Form Approved
OMB No. 2070-0106
Approval Expires 12-31-92

Please fill in blanks below.

Company Name	Company Number
Chemical Name	**EPA Chemical Number**

I Certify that:

My company is willing to develop and submit the data required by EPA under the authority of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), if necessary. However, my company would prefer to enter into an agreement with one or more registrants to develop jointly or share in the cost of developing data.

My firm has offered in writing to enter into such an agreement. That offer was irrevocable and included an offer to be bound by arbitration decision under section 3(c)(2)(B)(iii) of FIFRA if final agreement on all terms could not be reached otherwise. This offer was made to the following firm(s) on the following date(s):

Name of Firm(s)	Date of Offer

Certification:

I certify that I am duly authorized to represent the company named above, and that the statements that I have made on this form and all attachments therein are true, accurate, and complete. I acknowledge that any knowingly false or misleading statement may be punishable by fine or imprisonment or both under applicable law.

Signature of Company's Authorized Representative	Date
Name and Title (Please Type or Print)	

United States Environmental Protection Agency
Washington, D.C 20460

CERTIFICATION WITH RESPECT TO DATA COMPENSATION REQUIREMENTS

Form Approved
OMB No. 2070-0106
Approval Expires 12-31-92

Public reporting burden for this collection of information is estimated to average 15 minutes per response, including time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding the burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Chief, Information Policy Branch, PM-223, U.S. Environmental Protection Agency, 401 M. St., S.W., Washington, D.C. 20460; and to the Office of Management and Budget, Paperwork Reduction Project (2070-0106), Washington, D.C. 20503.

INSTRUCTIONS

Please fill in blanks below.

Company Name_____ Company Number_____

Chemical Name_____ EPA Chemical Number_____

I Certify that:

1. For each study cited in support of reregistration under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) that is an exclusive use study, I am the original data submitter, or I have obtained the written permission of the original data submitter to cite that study.

2. That for each study cited in support of reregistration under FIFRA that is NOT an exclusive use study, I am the original data submitter, or I have obtained the written permission of the original data submitter, or I have notified in writing the company(ies) that submitted data I have cited and have offered to: (a) Pay compensation for those data in accordance with section 3(c)(1)(D) and 3(c)(2)(D) of FIFRA; and (b) Commence negotiation to determine which data are subject to the compensation requirement of FIFRA and the amount of compensation due, if any. The companies I have notified are: (check one)

[ ] All companies on the data submitters' list for he active ingredient listed on this form (Cite-All Method or Cite-All option under the Selective Method). (Also sign the General Offer to Pay below.)

[ ] The companies who have submitted the studies listed on the back of this form or attached sheets, or indicated on the attached "Requirements Status and Registrants' Response Form," upon which I am relying in support of reregistration. (Selective Method)

3. That I have previously complied with section 3(c)(1)(D) of FIFRA for the studies I have cited in support of reregistration under FIFRA.

_____ _____
Signature Date

_____
Name and Title (Please Print)

GENERAL OFFER TO PAY: I hereby offer and agree to pay compensation to other persons, with regard to the reregistration of my products, to the extent required by FIFRA section 3(c)(1)(D) and 3(c)(2)(D).

_____ _____
Signature Date

_____
Name and Title (Please Print)

SUMMARY OF DATA FOR CHEMICAL SELECTION

Cedarwood Oil
8000-27-9

BASIS OF NOMINATION TO THE CSWG

Cedarwood oil is brought to the attention of the Chemical Selection Working Group (CSWG) as the active component of widely used insect repellants.

Three distinct cedarwood oil products, Virginia cedarwood oil, Texas cedarwood oil, and Western red cedar (*Thuja plicata*) oil, can be identified. The common major ingredients in the Virginia and Texas oils are cedrol, ∀-cedrene, and thujopsene, but the relative percentages vary depending on the origin of the cedar trees used to produce the oil. Western cedarwood oil contains methyl thujate and thujic acid.

Virginia cedarwood oil is widely used as a fragrance in soaps, air fresheners, household detergents, and cosmetics. The National Institute for Occupational Safety and Health (NIOSH) estimated that nearly 118 thousand workers are potentially exposed to Virginia cedarwood oil based on data collected in the 1980s. It is also the active ingredient in cedar balls/wood blocks used as moth repellants and in bug blocks. Because of concerns about the toxicity of naphthalene ("moth balls") and high concentrations of Deet (active ingedient in many bug blocks), the market for cedarwood oil products is expected to grow.

Although cedarwood oil has been described as a powerful abortifacient, very little data on the toxicity of any of the three cedarwood oils was found in a review of the available literature. Cedar shavings used as bedding have been reported to stimulate drug-metabolizing enzymes in rodents and affect the mortality of rat pups.

SELECTION STATUS

ACTION BY CSWG: June 20, 2002

Studies requested:

- Subchronic study (90 day) of Virginia cedarwood oil

Priority: Moderate

Rationale/Remarks:

- Widespread exposure to Virginia cedarwood oil even though production volumes are greater for Texas cedarwood oil, which has greater usage as a chemical intermediate.

- Potential substitute for naphthalene moth balls

- Lack of basic toxicology data on this product

-Reregistration eligibility has already been determined by EPA; additional toxicological data will not become available through this avenue.

INPUT FROM GOVERNMENT AGENCIES/INDUSTRY

Dr. Esther Rinde from the US Environmental Protection Agency (EPA) provided information on the status of testing required by the Reregistration Eligibility Decision (RED) for cedarwood oil.

CHEMICAL IDENTIFICATION

Cedarwood oils are extracted from several members of the family Cupressaceae, which includes true cedars, junipers, and cypresses. In the US, cedarwood oil is harvested mainly from *Juniperus virginiana* (Eastern red cedar or Virginia cedar), *Juniperus ashei* or *mexicana* (Texas cedar), and *Thuja plicata* (Western red cedar). According to the Food and Agriculture Organization of the United Nations (FAO), Western red cedar is the least used of the three (FAO, 1995a & 1995b).

Cedarwood oil, **Virginia**

CAS Registry Number:	8000-27-9
Chemical Abstracts Service Name:	Cedarwood oil, Virginiana (Allured FFM, 1999; ChemID, 2002). CAS registry number also applied to Chinese cedarwood oil (*Cupressus funebris*), Kenyan or East African cedarwood oil (*Juniperus procea*), and Moroccan or Atlas cedarwood oil (*Cedrus atlantica*) (ChemID, 2002; FAO, 1995a)
Synonyms and Trade Names:	Cedar oil; cedarwood oil; red cedarwood oil; EPA Pesticide Chemical Code 040505 (ChemID, 2002)

Chemical and Physical Properties:

Description:	Light yellow to pale brown viscous liquid; sometimes solidifies at room temperature; cedar odor (Gerhartz, 1988)
Density:	d^{25} 0.939-0.958 (Gerhartz, 1988)
Solubility:	Soluble in ethanol (Gerhartz, 1988)

Texas Cedarwood oil

Prepared for NCI by Technical Resources International, Inc. to support nomination under contract no. N02-CB-07007 (CSWG:05/02; NTP: 9/02)

CAS Registry Number: 68990-83-0

Chemical Abstracts Service Name: Texas cedarwood oil (Allured FFM, 1999; ChemID, 2002)

Synonyms and Trade Names: Texan cedarwood oil (ChemID, 2002)

Chemical and Physical Properties:

Description: Brown to reddish-brown, viscous liquid; may partially solidify at room temperature; cedar odor(Gerhartz, 1988)

Density: d^{20} 0.954-0.967 (Gerhartz, 1988)

Solubility: Soluble in ethanol (Gerhartz, 1988)

Western red cedar oil

CAS Registry Number: 68917-35-1

Chemical Abstracts Service Name: *Thuja plicata* oil (STNEasy, 2002)

Synonyms and Trade Names: Western red cedarwood oil (Laurel Laboratories, Inc., 2002)

Cedarwood oil components: The composition of cedarwood oils varies depending on the source. Cedrol and thujopsene are the major components of Texas and Virginia oils; Virginia oils also contain significant quantities of ∀-cedrene (Mookherjee & Wilson, 1996). The volatile oil from Western juniper has been reported to contain 15-40% cedrol (Kurth & Ross, 1954). Methyl thujate and thujic acid are the primary ingredients found in Western red cedarwood oil (Laurel Laboratories, Inc., 2002). The principal constituents of cedarwood oils are shown in Table 1.

Table 2 summarizes the chemical and physical properties of the major components of Virginia and Texas cedarwood oils.

Table 1. Chemical Composition of Cedarwood Oils

Component	CAS No.	Texas Oil (%)	Virginia Oil (%)	Western Red Cedarwood Oil (%)
Thujopsene	470-40-6	60.4	27.6	-
Cedrol	77-53-2	19.0	15.8	-
∀-Cedrene	469-61-4	1.8	27.2	-
∃-Cedrene	546-58-1	1.6	7.7	-
∀-Copaene	3856-25-5	2.8	6.3	-
Widdrol	6892-80-4	1.1	1.0	-
Methyl thujate		-	-	65
Thujic acid		-	-	25
∃-Thujaplicin	499-44-5	-	-	1
∀-Thujaplicin		-	-	1

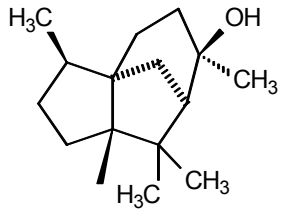
Source: Lawrence, 1993; Laurel Laboratories, Inc., 2002; Mookherjee & Wilson, 1996

Technical Products and Impurities: The composition of cedarwood oils is complex and varies depending on the species of trees used in the extraction process. The International Organization for Standardization (ISO) and the Fragrance Manufacturers Association (FMA) provides standards for Texas and Virginia cedarwood oils. The ISO standard specifies an alcohol content (expressed as cedrol) of 35-48% with a minimum cedrol content of 20% for Texas cedarwood oil. For Virginia oil, a maximum cedrol content of 14% is specified. Compositional data for American oils, as specified by the FMA standards are somewhat different. The FMA standard specifies the alcohol content

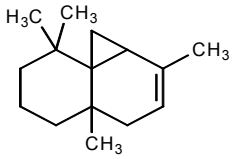
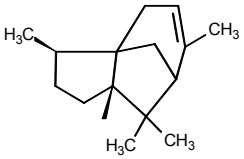
(cedrol and related isomers) for Texas oils as 25-42% and for Virginia oils as 18-38% (FAO, 1995a).

Chinese and *Cedrus atlanticus* cedarwood oil imports are also commercially available (AromaWeb, 2001; Schreiber, 1996). Chinese cedarwood oil from *Cupressus funebris*, which is widely used in the US, has a composition very similar to Texas cedarwood oil (Gerhartz, 1988; Schreiber, 1996). The FMA requires an 8% minimum alcohol content for Chinese cedarwood oil (FAO, 1995a).

Table 2. Chemical and Physical Properties of Major Components of Virginia and Texas Cedarwood Oils

Component	Synonyms	Chemical and Physical Properties/Percentage in oil
Cedrol [CAS No. 77-53-2]  Mol. Wt.: 222.37 $C_{15}H_{26}O$	(+)-Cedrol (ChemID, 2002)	Crystalline solid, needles from dil. methanol (Merck, 2001; Sigma-Aldrich, 2002a) Melting Point: 86-87 EC (Merck, 2001)

Prepared for NCI by Technical Resources International, Inc. to support nomination under contract no. N02-CB-07007 (CSWG:05/02; NTP: 9/02)

Thujopsene [CAS No. 470-40-6]  Mol. Wt.: 204.35 $C_{15}H_{24}$	(-)-Thujopsene, widdrene (ChemID, 2002)	Colorless, clear liquid (Merck, 2001; Sigma-Aldrich, 2002a) Boiling Point: 120EC (Merck, 2001); 258-260 EC (Sigma-Aldrich, 2002a) Flash Point: 104EC (Sigma-Aldrich, 2002a) Solubility: Insoluble in water, soluble in common organic solvents (Sigma-Aldrich, 2002a)
∀-Cedrene [CAS No. 469-61-4]  Mol. Wt.: 204.36 $C_{15}H_{24}$	Cedr-8-ene, (ChemID, 2002)	Colorless, clear liquid (Sigma-Aldrich, 2002a) Boiling Point: 261-262EC (Sigma-Aldrich, 2002a) Flash Point: 104EC (Sigma-Aldrich, 2002a) Solubility: Insoluble in water, soluble in benzene (Sigma-Aldrich, 2002a)

Virginia cedarwood oil [CAS No. 8000-27-9], Texas cedarwood oil [CAS No. 68990-83-0], (+)-cedrol (∃99.0 %) [CAS No. 77-53-2], (-)-thujopsene (∃97.0 %) [CAS No. 470-40-6], (-)-∀-cedrene (∃99.0 %) [CAS No. 469-61-4], (+)- ∃-cedrene (~97 %) [CAS No. 546-28-1], (-)-∀-copaene (~95 %) [CAS No. 3856-25-5], and ∃-thujaplicin (99%) [CAS No. 499-44-5] are available from Sigma-Aldrich (Sigma-Aldrich, 2002b).

Prepared for NCI by Technical Resources International, Inc. to support nomination under contract no. N02-CB-07007 (CSWG:05/02; NTP: 9/02)

EXPOSURE INFORMATION

Production and Producers:

Manufacturing Process: Virginia cedarwood oil is produced by steam distillation of sawdust, finely chipped waste wood from the manufacture of cedarwood products, or from stumps and logs of *Juniperus virginiana*. Texas cedarwood oil is obtained by steam distillation of chopped wood from *Juniperus ashei* or *Juniperus mexicana*. Although the most popular method for precessing cedarwood for oil production is steam distillation, some cedarwood oils are also produced through solvent extraction (Australian National University, 1999; Gerhartz, 1988; Schreiber, 1996).

Producers and Importers: Thirteen US producers or distributors of cedarwood oil are listed by Chemical Sources International (2002).

According to recent issues of chemical directories, cedarwood oil is manufactured and/or distributed by Berje Inc.; Brutanicals, Inc.; The Lebermuth Co., Inc.; Penta Manufacturing Co.; Polarome International, Inc.; Ruger Chemical Co., Inc.; and Spectrum Chemical MFG Corp. (Hunter, 2001; Tilton, 2001).

Production/Import/Export Level: Virginia and Texas cedarwood oils, *Thuja plicata* oil, cedrol, ∀-cedrene, and thujopsene are listed in the EPA Toxic Substances Control Act (TSCA) Inventory (ChemID, 2002).

The Port Import/Export Reporting Service (PIERS) reported cedarwood oil imports with a cargo weight of 23,302 pounds over the 11 month period from April 3, 2001 to February 20, 2002. For the 18-month period between September 12, 2000 and March 16, 2002, PIERS reported cedarwood oil exports with a cargo weight of 96,012 pounds (Dialog Information Services, 2002a, 2002b).

Use Pattern: The US production of cedarwood oil in 1984 was reported to be 1,400 tons for the Texas oil and 240 tons for the Virginia oil (FAO, 1995b). The production of cedarwood oil is expected to rise because of government incentive programs for the use of red cedar trees (Adams, 1987; Oklahoma State University, 2001).

Although Texas cedarwood oil is produced in larger quantities than cedarwood oil from the Eastern cedar *(Juniperus virginiana)*, it is used almost exclusively as feedstock for the manufacture of chemical derivatives, such as cedrol, cedryl methyl ether, acetyl cedrene, and cedryl acetate. Imports of Chinese cedarwood oil are also used to produce these derivatives. In contrast, Virginia cedarwood oil is widely used in the fragrance industry, among others (FAO, 1995b; Gerhartz, 1988; Schreiber, 1996).

Virginia cedarwood oils have many commercial uses. They are used to restore the smell of cedar to furniture and in cosmetic formulations, including shampoos for humans and animals, aftershave lotions, soap bars, and perfumes. They are also found in insect repellents, massage oils, incense oils, and shavings used as bedding for small animals. *Thuja plicata* or *Cedrus atlantica* cedarwood oils may also be used in some of these products (Absorbine Jr., 2002; Adams, 1987; Australian National University, 1999; Aroma-essence.com, 2002; Cedarcide, 2002; Country Cottage Works, 2002; Drugstore.com, 2002; FAO, 1995b; Frontier Natural Products Co-op, 2002; Lady Lorelei, 2002; POCO, LLC, 2002; Resource Management Group, 2002; Sawyer, 2002; Skeeter Defeater, 2002).

Cedarwood oil alcohols and terpenes are food additives considered by the US Food and Drug Administration (FDA) to be Generally Recognized as Safe (GRAS). These

Prepared for NCI by Technical Resources International, Inc. to support nomination under contract no. N02-CB-07007 (CSWG:05/02; NTP: 9/02)

food additives are used as flavor enhancers, flavoring agents, or adjuvants. Food use of cedarwood oil terpenes was estimated to be 166,666 lb in 1987 (EPA, 1993; FDA, 2002; Clydesdale, 1997).

Cedarwood oil and thickened cedarwood oil have laboratory uses as immersion oils for light microscopy and for clearing microscope sections (Baker's Chemicals, 2002; PolySciences, Inc., 2001; Sigma-Aldrich, 2002b).

Cedarwood oil, in combination with other products, is used as a homeopathic remedy and is sold as a vaporizing ointment for topical use (Health Canada, 2002).

A total of 349 patents using cedarwood oil were on file with the US Patent and Trademark Office (USPTO) as of May 2002 (US Patents and Trademark Office, 2002).

Human Exposure:

Occupational Exposure: The National Occupational Exposure Survey (NOES), which was conducted by the National Institute for Occupational Safety and Health (NIOSH) between 1981 and 1983, estimated that 117,858 workers in 7,990 facilities representing 67 industries were potentially exposed to Virginia cedarwood oil in the workplace. The NOES database does not contain information on the frequency, level, or duration of exposure to workers of any chemical listed therein (Sigma-Aldrich, 2002a).

Cedarwood oil exposure may also occur in laboratories as a result of its use as immersion oil (PolySciences, 2001).

Environmental Exposure: ∀-Cedrene, a constituent of cedarwood oil, has been found in chemical wastes in the water of the Great Lakes. The sources of ∀-cedrene may derive from natural plant products or pulp mills (Passino-Reader *et al*., 1997).

Consumer Exposure: The largest number of human exposures to cedarwood oil occurs among consumers using insecticide products, deodorants, soaps, air fresheners, floor polishes, and sanitation supplies containing cedarwood oil. Consumers are potentially exposed to cedarwood oil through inhalation and dermal contact.

Although registration as a pesticide is no longer required by EPA for cedarwood oil, several formulations have been registered as pesticides in the past. These formulations include: ready-to-use liquids containing 0.48% cedarwood oil, wood blocks containing 2-8% cedarwood oil, and pet collars with 0.5% cedarwood oil (EPA, 1993). Two pesticides, Bug Block sunscreen and insect repellent, containing 0.46% Texas cedarwood oil, and Nexa cedarwood oil moth protection, containing 40% Virginia cedarwood oil, are registered with the state of California as pesticides (California EPA, 2001).

Two typical products for human topical use, Cedarcide (2002) and Skeeter Defeater (2002) contain 1 and 5% cedarwood oil, respectively.

Environmental Occurrence: Cedarwood oil contains natural products found in cedar, juniper, and cypress woods and steam distillation derivatives of such products.

Regulatory Status: No standards or guidelines have been set by NIOSH or the Occupational Safety and Health Administration (OSHA) for occupational exposure to or workplace allowable levels of cedarwood oil. Cedarwood oil is not listed on the American

Prepared for NCI by Technical Resources International, Inc. to support nomination under contract no. N02-CB-07007 (CSWG:05/02; NTP: 9/02)

Conference of Governmental Industrial Hygienists (ACGIH) list of compounds for which recommendations for a Threshold Limit Value (TLV) or Biological Exposure Index (BEI) are made.

Cedarwood oil was initially registered in 1960 as a pesticide to repel moths from clothing. As such, cedarwood oil was subject to the requirements for reregistration eligibility promulgated in 1988. EPA issued its Reregistration Eligibility Decision (RED) document for cedarwood oil in 1993. The RED profiles the use of cedarwood oil as a natural repellant/feeding depressant and fungicide used in houses and on pets or their bedding to repel fleas, moths, and mildew. Based on information collected as the result of the RED, EPA deregulated cedarwood oil in 1996 and no longer requires manufacturers of cedarwood oil products to register them as pesticides (EPA, 1993; Rinde, 2002).

Cedarwood oil is not listed as a hazardous substance, priority pollutant, or toxic pollutant under the Clean Water Act.

EVIDENCE FOR POSSIBLE CARCINOGENIC ACTIVITY

Human Data: No epidemiological studies or case reports investigating the association of exposure to cedarwood oil and cancer risks in humans were identified in the available literature.

Reports describing cedarwood oils as irritants or sensitizers appear to be rare (Botanical Dermatology Database, 2001). Cedarwood oil (no origin specified) produced no response in humans after 48 h when administered as a patch test at a concentration of 8% in petrolatum (HSDB, 2002). The Danish Environmental Protection Agency (2002) classified cedarwood oil used in household and cosmetic detergents as a rare sensitizer fragrance based on the results of patch tests on patients with cosmetic dermatitis and controls.

Historically, nineteenth century medical compendiums contain several reports of abortion and death in humans after oral consumption of relatively large amounts of cedarwood oil (Allen, 1877; HSDB, 2002).

Animal Data: No 2-year carcinogenicity studies of cedarwood oil or its components in animals were identified in the available literature.

The LD_{50} values for Virginia cedarwood oil and cedrol are given in Table 3.

Table 3. Acute Toxicity Values for Cedarwood Oil and Cedrol

Compound	Species	Route of administration	LD_{50} (g/kg)
Virginia cedarwood oil	rat	oral	>5
Virginia cedarwood oil	rabbit	dermal	>5
Cedrol	rabbit	dermal	>5

Source: Sigma-Aldrich (2002a)

Several limited studies reported a possible correlation between the use of cedarwood bedding and an increased incidence of spontaneous cancer in animals:

- American-born C3H-A^{vy} and C3H-A^{vy}fB mice raised in the US have nearly a 100% incidence of liver and mammary tumors. These strains, bred and reared in Australia on sawdust bedding from Douglas fir, had almost no spontaneous incidence of mammary and liver tumors, particularly after the first generation. In contrast, virtually all C3H-A^{vy} mice reared in Australia but kept on US bedding (cedar) and fed US diets developed mammary tumors. The authors expressed their opinion that the cedar appeared to be the "carcinogenic" agent, noting that the results involved a limited number of animals (Sabine *et al.*, 1973).

- Sabine (1975) conducted follow-up studies to further examine the role of cedar bedding vs other parameters on the spontaneous incidence of tumors in the susceptible strains of mice. Sabine concluded that:

  > Based on data accumulated over 5 years, the incidences of mammary tumors and hepatomas in three strains of mice (C3H-A^{vy}, C3H-A^{vy}fB, and CBA/J) housed on Douglas fir sawdust bedding were significantly lower than the reported figures from US laboratories.
  >
  > Following submission of his paper, Sabine became aware of a publication by Dr. Heston, of the US National Cancer Institute (NCI). Heston had provided the Australian investigators with their initial colony of C3H-A^{vy} and C3H-A^{vy}fB mice. When Heston bedded two groups of mice on either ¾ pine sawdust and ¼ cedar shavings or pine sawdust, both groups developed very high incidences of spontaneous mammary tumors and hepatomas. Heston attributed the lower incidences of spontaneous tumors seen in the Australian study to higher ectoparasite infestations and slightly lower growth rates (Heston, 1975).

Burkhart and Robinson (1978) described a high rate of rat pup deaths, which the authors felt was probably caused by Eastern cedarwood bedding, either through ingestion of inhalation of toxic compounds in the bedding or through the milk of the dams.

Vlahakis (1977) reported that the first generation of C3H-A^{vy}fB crossbred mice had the same high incidences of mammary and liver tumors whether they were raised using pine bedding or a mixture of pine plus red cedar shavings.

Short-Term Tests: No *in vitro* or *in vivo* studies evaluating cedarwood oil or its components for mutagenic activity were found in the available literature.

Other Biological Effects:

Hexobarbital Sleeping Time: Housing the animals using cedarwood bedding resulted in a highly significant reduction of hexobarbital sleeping time in C3H-A^{vy}, CBA/J, and Swiss Albino mice, indicating induction of the enzymes responsible for hexobarbital oxidation. Using the same methodology, the authors demonstrated enzyme induction in CBA/J mice from Virginia cedarwood oil (Sabine, 1975).

The increase in the duration of hexobarbital hypnosis following exposure of Swiss-Webster mice to cedar shavings was previously reported by Wade and coworkers at the University of Georgia. These investigators then exposed mice to various fractions of cedarwood for up to 10 days and measured the duration of hexobarbital anesthesia, which suggested that cedrol and cedrene were the causative agents (Wade et al., 1968).

Insecticidal Properties: Cedarwood shavings from *Juniperus virginiana* arrested the life cycle at the 1st instar stage of the Peanut Trash Bug (*Elasmolomus sordidus*). It also

Prepared for NCI by Technical Resources International, Inc. to support nomination under contract no. N02-CB-07007 (CSWG:05/02; NTP: 9/02)

caused the death of colonies of Indian Moths (*Plodia interpunctella*) and Forage Mites (*Tyrophagus putrescentiae*). Virginia cedarwood oil (3%), cedrene (2%), and cedrol (2%) were all highly toxic to Peanut Trash Bug colonies. Cedarwood oil and cedrene also affected the reproductive behavior of adults or hatchability of eggs. Colonies of German cockroaches (*Blatella germanica*) were not affected by cedarwood from *Juniperus virginiana* (Sabine, 1975).

Components of cedarwood oils:

$ Cedrene prevented the butylated hydroxytoluene (BHT)-induced inhibition of lung tumors caused by intraperitoneal injection of urethan in strain A mice (Malkinson & Beer, 1984).

$ The effect of cedrene on *in vitro* hepatic metabolism was studied in Sprague-Dawley rats. Administration of cedrene using the oral, intraperitoneal and inhalation routes, increased the ethylmorphine *N*-demethylase activity and cytochrome P-450 content, while it had no effects on aniline hydroxylase activity (Hashimoto *et al*., 1972).

$ ∃-Thujaplicin, a compound found in the heartwood of the western red cedar (*Thuja plicata*), was teratogenic when administered to ICR mice at very high doses. *In vitro*, ∃-thujaplicin induced growth retardation and malformation of cultured embryos harvested at 9 days of gestation. *In vivo*, 420-1,000 mg/kg of ∃-thujaplicin, given orally to pregnant ICR mice on day 9 of gestation, induced cleft palates and lips, facial dysmorphism, and other malformations at doses of 560 mg/kg or above in 18-d old fetuses. The oral LD_{50} for ∃-thujaplicin was 750-800 mg/kg (Ogata *et al.*, 1999).

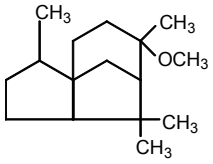
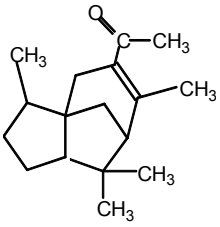
Prepared for NCI by Technical Resources International, Inc. to support nomination under contract no. N02-CB-07007 (CSWG:05/02; NTP: 9/02)

- Ecotoxicological studies on ∀-cedrene showed that the EC_{50} was 0.044 mg/L at 48 h for *Daphnia pulex* (Passino-Reader *et al*., 1997).

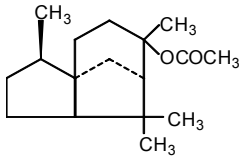
Structure Activity Relationships:

Three derivatives of the major chemical constituents of cedarwood oil were selected for review. These chemicals were cedrol methyl ether [CAS No.67874-81-1], acetyl cedrene [CAS No.80449-58-7], and cedryl acetate [CAS No. 77-54-3]. No information on the carcinogenicity or genotoxicity of these compounds in a search of the National Library of Medicine TOXNET databases, including TOXLINE. No information on any of these chemicals was located in the 1999 version of CancerChem, the CD-ROM version of NCI's *Survey of Compounds Which Have Been Tested for CarcinogenicomActivity* (PHS-149), available from GMA Industries, Inc.

Table 4. Information on Derivatives of the Major Components of Cedarwood Oil.

Compound	Structure	Uses
Cedrol methyl ether CAS No.: 67874-81-1		Fragrance in cosmetics (Allured FFM, 1999; Gerhartz, 1988)
Acetyl cedrene CAS No.: 80449-58-7		Fragrance (Allured FFM, 1999; Bledsoe, 1997)

Prepared for NCI by Technical Resources International, Inc. to support nomination under contract no. N02-CB-07007 (CSWG:05/02; NTP: 9/02)

Cedryl acetate CAS No.: 77-54-3		Fragrance in perfume, fixative, food additive (Allured FFM, 1999; FDA, 2002; Gerhartz, 1988)

Prepared for NCI by Technical Resources International, Inc. to support nomination under contract no. N02-CB-07007 (CSWG:05/02; NTP: 9/02)

References

Absorbine Jr. (2002) Bug Block®. *Absorbine Jr.® Products*. [http://www.absorbinejr.com/ products/ bug_block.htm]. Searched May 7, 2002

Adams, R.P. (1987) Investigation of *Juniperus* species of the United States for new sources of cedarwood oil. *Econ. Bot.*, **41**(1), 48-54

Allen, T. (1877) *The Encyclopedia of Pure Materia Medica*, vol. 5 Hyd-Lyc, Philadelphia, PA, Boericke & Tafel. [http://www.homeoint.org/allen/juni.htm]. Searched December 17, 2001

Allured FFM (1999) Cedarwood oil, cedrol, cedrene, cedryl acetate, acetylcedrene. Allured's Flavor and Fragance Materials, ed. (On CD-ROM), Carol Stream, IL, Allured Publishing Corp.

Aroma-essence.com (2002) *Kidscents*. [http://www.aroma-essence.com/catalog/kidscents.html]. Searched May 5, 2002

AromaWeb (2001) Atlas cedarwood essential oil. *E.O. Profiles*. [http://www.aromaweb.com/ essentialoilsaf/atlascedarwood.asp]. Searched December 17, 2001

Australian National University (1999) *Cedarwood oil: it's history, harvesting, processing, yield, and uses*. The Australian National University. [http://sres.anu.edu.au/associated/fpt/nwfp/ cedarwood/cedarwood.html]. Searched May 5, 2002

Baker's Chemicals (2002) Results of Search for: cedarwood oil. *Baker's Chemicals*. Virtual Scientific Industries. [http:www.virtual-scientific.com/Catalog/products/chemicals/ chemicals1.htm]. Searched May 17, 2002

Bledsoe, J.O. (1997) Terpenoids. In: Krokshwitz, J.I. & Howe-Grant, M., eds., *Kirk-Othmer Encyclopedia of Chemical Technology*, 4th ed., vol. 23, New York, John Wiley & Sons, Inc., p 839

Botanical Dermatology Database (2001) Cuppressaceae (Cypress family). *Botanical Dermatology Database*. [http://216.51.100/]. Searched May 2, 2002

Burkhart, C.A. & Robinson, J.L. (1978) Hugh rat pup mortality attributed to the use of cedar-wood shavings as bedding. *Lab. Animals*, **12**, 221-222

California EPA (2001) California Environmental Protection Agency Department of Pesticide Regulation, *USEPA/OPP Pesticide Related Database Queries: Cedarwood oil.*

[http://www.cdpr.ca.gov/]. Searched December 19, 2001

Cedarcide (2002) Chemical free, 100% natural pest control. *Cedarcide*. [http://www. pestproducts. com/cedarcide.htm]. Searched May 1, 2002

Chemical Sources International (2002) *All Chemical Suppliers for: Cedarwood oil* [http://db/chemsources.com]. Searched May 16, 2002

ChemID (2002) Cedarwood oil, cedarwood oil Texas, *Thuja plicata* oil, cedrol, alpha-cedrene, thujopsene, acetyl cedrene, cedrol methyl ether, 3-thujopsanone. *ChemIdplus*. National Library of Medicine, Bethesda, MD. Records No. 008000279, 068990830, 068917351, 000077532, 000469614, 000470406, 080449587, 125783655, 067874811, 025966794. [http://chem.sis.nlm. nih.gov/chemidplus/]. Searched May 9 and 13, 2002

Clydesdale, F. M., ed. (1997) Cedarwood oil alcohols. *Food Additives. Toxicology, Regulation, and Properties.* (On CD-ROM), Boca Raton, FL, CRC Press, Inc.

Country Cottage Works (2002) Country Cottage Works aromatherapy blends! *Blended Oils.* [http://www.countrycottageworks.com/boil.htm]. Searched May 22, 2002

Danish Environmental Protection Agency (2002) *Environmental and Health Assessment of Substances in Household Detergents and Cosmetic Detergent Products.* [http://www.mst.dk/ udgiv/publications/ 2001/87-7944-596-9/html/kap12_eng.htm]. Searched May 5, 2002

Dialog Information Services (2002a) *PIERS Imports (US Ports) (File 573)*, Palo Alto, CA. Searched April 25, 2002 [Accession Nos. 0024967128, 0023643954, 0023091615, 0021582468, 0020784810, 0019469008]

Dialog Information Services (2002b) *PIERS Exports (US Ports) (File 571)*, Palo Alto, CA. Searched Searched April 25, 2002 [Accession Nos.0024038447, 0023971590, 0023952622, 0023871459, 0018773530, 0018713411, 0018711860, 00186903233, 0018653985, 0018652219, 0018638069]

Drugstore.com (2002) *Dove. Beauty Bar, sensitive skin*. [http://www. drugstore.com]. Searched May 3, 2002

EPA (1993) *Reregistration Eligibility Decision (RED) Cedarwood Oil*. Washington, DC, US Environmental Protection Agency, EPA 738-S-93-012, 169 pp

FAO (1995a) *Flavours and Fragances of Plant Origin*. Food and Agriculture Organization of

the United Nations. [http://www.fao/org/docrep/V5350e/V5350e00.htm]. Searched May 2, 2002

FAO (1995b) *Non-Wood Forest Products from Conifers.* Food and Agriculture Organization of the United Nations. [http://www.fao/org/docrep/x0453e/X0453e00.htm]. Searched May 2, 2002

FDA (2002) "Everything" Added to Food in the United States. *EAFUS: a Food Additive Database*. US Food and Drug Administration. [http://www.cfscan.fda.gov/~dms/eafus.html]. Searched May 8, 2002

Frontier Natural Products Co-op (2001) Red cedarwood essential oil. *Aromatherapy Pat's Picks*. [http://www.frontierherb.com/Aromatherapy/AromaNotes/Note12.html]. Searched December 17, 2001

Gerhartz, W., ed. (1988) Flavors and Fragrances. In: Elvers, B., Rousanville, J.F., & Schulz, G., eds., *Ullmann's Encyclopedia of Industrial Chemistry*, Vol. A11, VCH Publishers, p. 171, 176, 177, 219, 220

Hashimoto, M., Davis, D.C. & Gillette, JR. (1972) Effect of different routes of administration of cedrene on hepatic drug metabolism. *Biochem. Pharmacol.*, **21**, 1514-1517

Health Canada (2002) Cedarwood oil. *Drug Product Database*. [http://www.hc-sc.gc.ca/hpb/drugs-dpd/]. Searched May 5, 2002

Heston, W.E. (1975) Testing for possible effects of cedar wood shavings and diet on occurrence of mammary gland tumors and hepatomas in C3H-A^{vy} and C3H-A^{vy}fB mice. *J. Natl. Cancer Inst*., **54**, 1011-1014

HSDB (2002) Cedarwood oil. *HSDB (Hazardous Substances Data Bank)*, National Library of Medicine, Bethesda, MD. [http://toxnet.nlm.nih.gov/]. Searched May 9, 2002

Hunter, D., ed. (2001) *Chemical Week 2001 Buyers' Guide*, New York, Chemical Week Associates, p. 190

Kurth, E.F. & Ross, J.D. (1954) Volatile oil from Western Juniper. Report No. C-3. Oregon Forest Products Laboratory. [http://juniper.orst.edu/kurth.htm]. Searched May 6, 2002

Lady Lorelei (2002) *Sense of Serenity. Woodland mists*. [http://www.lunarace.com/opinioa.html]. Searched May 1, 2002

Laurel Laboratories, Inc. (2002) *An Introduction to the Oil of Western Red Cedarwood Pacific*.. [http://home.istar.ca/~daijones/index.html]. Searched May 8, 2002

Lawrence, B.M., ed. (1993) Cedarwood oil. *Essential Oils* (1988-1991). Carol Stream, IL, Allured Publishing Corp., pp 176-177

Malkinson, A.M. & Beer, D.S. (1984) Pharmacologic and genetic studies on the modulatory effects of butylated hydrxytoluene on mouse lung adenoma formation. *J. Natl. Cancer Inst*., **73**(4), 925-933

Merck (2001) Thujopsene, Cedrol.9470, 1923. *The Merck Index*, 13th ed. (On CD-Rom), Rahway, NJ, Chapman & Hall [Monograph Numbers 1923 & 9470]

Mookherjee, B.D. & Wilson, R.A. (1996) Oils, essential. In: Krokshwitz, J.I. & Howe-Grant, M., eds., *Kirk-Othmer Encyclopedia of Chemical Technology*, 4th ed., vol. 17, New York, John Wiley & Sons, Inc., p 644-645

Ogata, A., Ando, H., Kubo, Y., Nagasawa, H., Ogawa, H., Yasuda, K. & Aoki, N. (1999) Teratogenicity of thujaplicin in ICR mice. *Food & Chem. Toxicol.*, **37**, 1097-1104

Oklahoma State University (2001) *Turning a problem into profit*. [http://www.okstate.edu/ag/impact/impact1.htm]. Searched December 17, 2001

Passino-Reader, D.R., Hickey, J.P. & Ogilvie, L.M. (1997) Toxicity to *Daphnia pulex* and QSAR predictions for polycyclic hydrocarbons representative of Great Lakes contaminants. *Bull. Environ. Contam. Toxicol.*, **59**, 834-840

POCO, LLC (2002) *Welcome to POCO, LLC*. Port Orford Cedar Oil, LLC. [http://12.32.114.93/Aboutus.htm]. Searched May 1, 2002

PolySciences, Inc. (2001) Result of Search for: cedarwood oil. *Online Catalog*. [http://www.polysciences.com/]. Searched May 17, 2002

Rinde, E. (2002) Personal communication (telephone conversation) from Esther Rinde, PhD, US Environmental Protection Agency, to Marta De Santis, PhD, Technical Resources International, May 7, 2002

Resource Management Group (2002) *Cedar Info*. [http://rmg.8k.com]. Searched May 6, 2002

Sabine, J.R. (1975) Exposure to an environment containing the aromatic red cedar, *Juniperus virginiana*: procarcinogenic, enzyme-inducing and insecticidal effects. *Toxicology*, **5**, 221-235

Sabine, J.R., Horton, B.J., and Wicks, M.B. (1973) Spontaneous tumors in C3H-A^{vy} and C3H-A^{vy}fB mice: high incidence in the United States and low incidence in Australia. *J. Natl. Cancer Inst.*, **50**, 1237-1242

Sawyer (2002) Insect repellent ingredients. *Products and Knowledge to Increase Your Outdoor Enjoyment.* [http://www.sawyerproducts.com/Insect_Repellent/repellents_ingredients.htm]. Searched May 6, 2002

Schreiber, W.L. (1996) Perfumes. In: Krokshwitz, J.I. & Howe-Grant, M., eds., *Kirk-Othmer Encyclopedia of Chemical Technology*, 4th ed., vol. 18, New York, John Wiley & Sons, Inc., p 183, 190

Sigma-Aldrich (2002a) Cedarwood oil, Virginia cedarwood oil, cedrol, ∀-cedrene, and thujopsene. *Material Data Sheets.* [http://www.sigma-aldrich/com]. Searched May 20, 2002

Sigma Aldrich (2002b) Cedarwood oil, Virginia cedarwood oil, Texas cedarwood oil, cedrol, ∀-cedrene, ∃-cedrene, ∀-copaene, thujopsene, and ∃-thujaplicin. *Sigma Aldrich Search Results.* [http://www.sigma-aldrich/com]. Searched May 20, 2002

Skeeter Defeater (2002) Cedarwood soap. *Skeeter Defeater*. [http://www.worldpromo.com/wholesale/cedarwood_soap.htm]. Searched May 1, 2002

STNEasy (2002) Result of Search for: *Thuja plicata* oil. Scientific & Technical Information Network, Chemical Abstracts Services, Inc. [http://stneasy.cas.org]. Searched May 15, 2002

Tilton, H., ed. (2001) Cedarwood oil. *OPD 2002 Chemical Buyers Directory*, New York, Schnell Publishing Co., p 180

US Patents and Trademark Office (2002) Results of search (all years) of database for cedarwood oil: 349 patents, *Welcome to the USPTO Web Patent Databases.* [http://www. uspto.gov/]. Searched May 17, 2002

Vlahakis, G. (1977) Possible carcinogenic effects of cedar shavings in bedding of C3H-A^{vy}fB mice. *J. Natl. Cancer Inst.*, **58**, 149-150

Wade, A.J., Holl, J.E., Hilliard, C.C., Molton, E. & Greene, F.E. (1968) Alteration of drug metablolism in rats and mice by an environment of cedarwood. *Pharmacology*, **1**, 317-328

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.

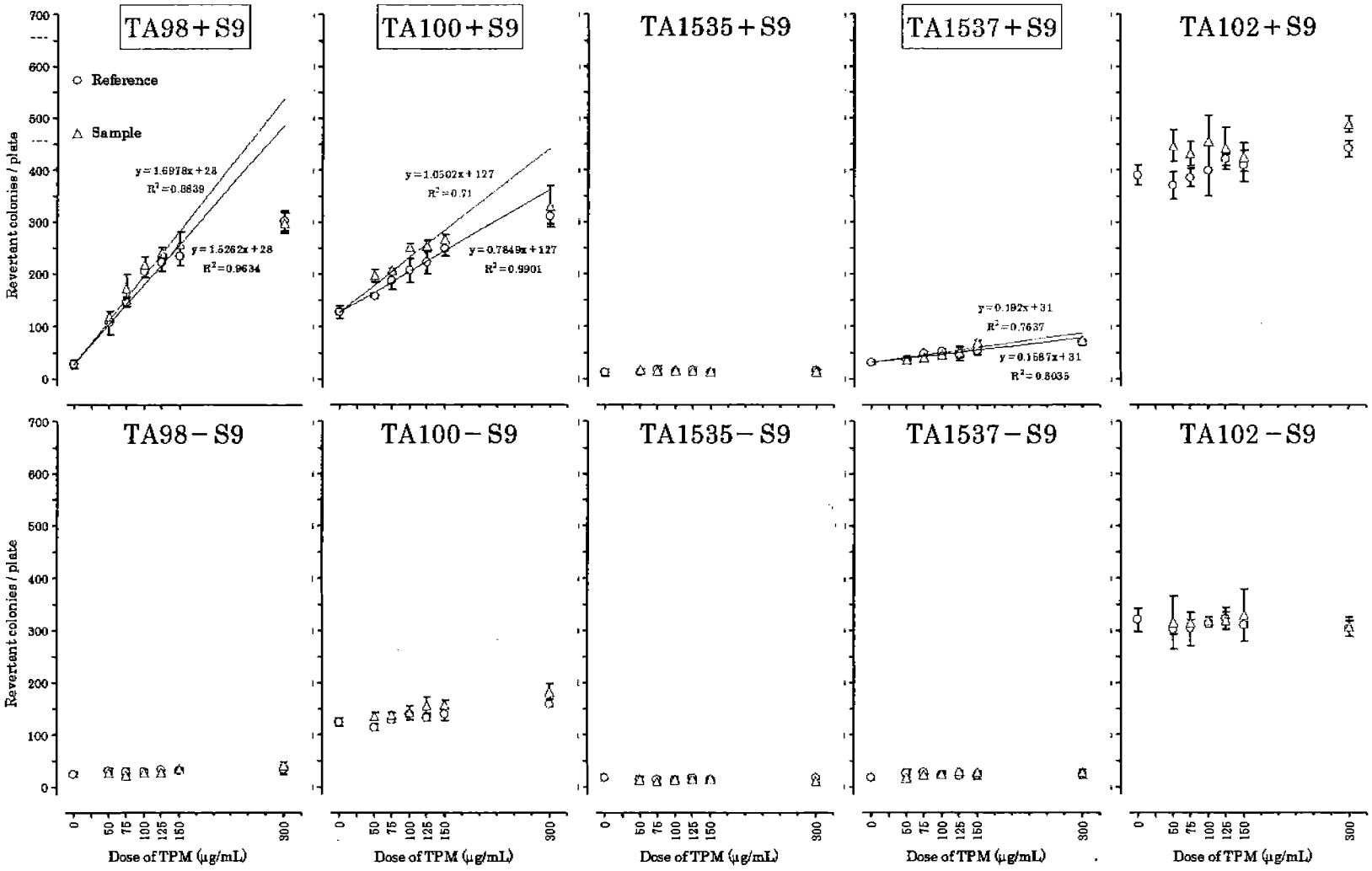
Body Weights and Clinical Observations

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.

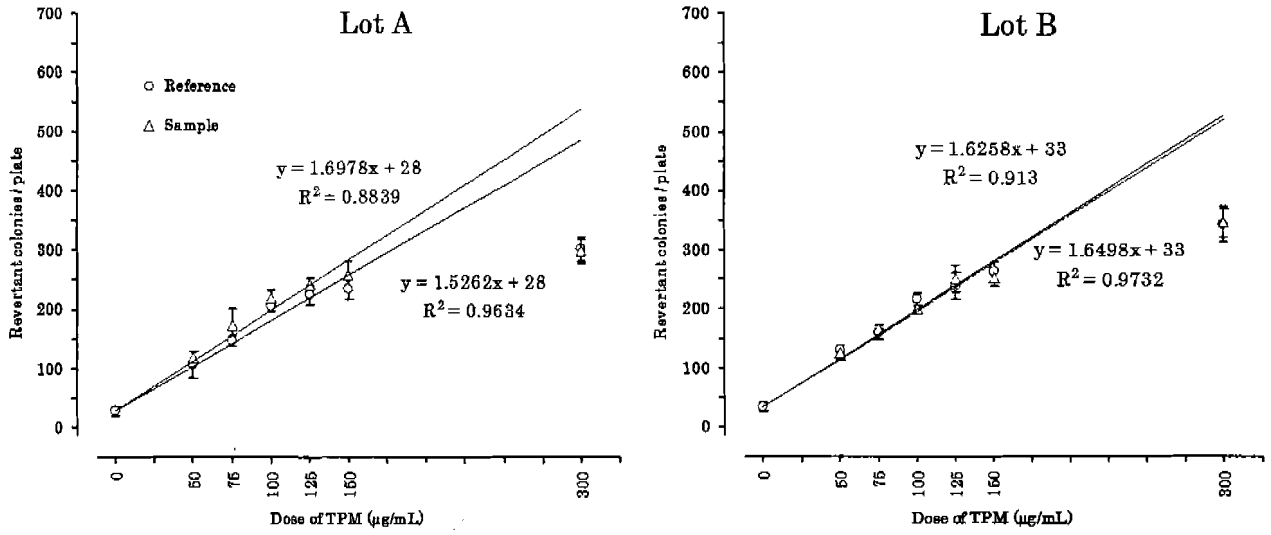
Organ Weights

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

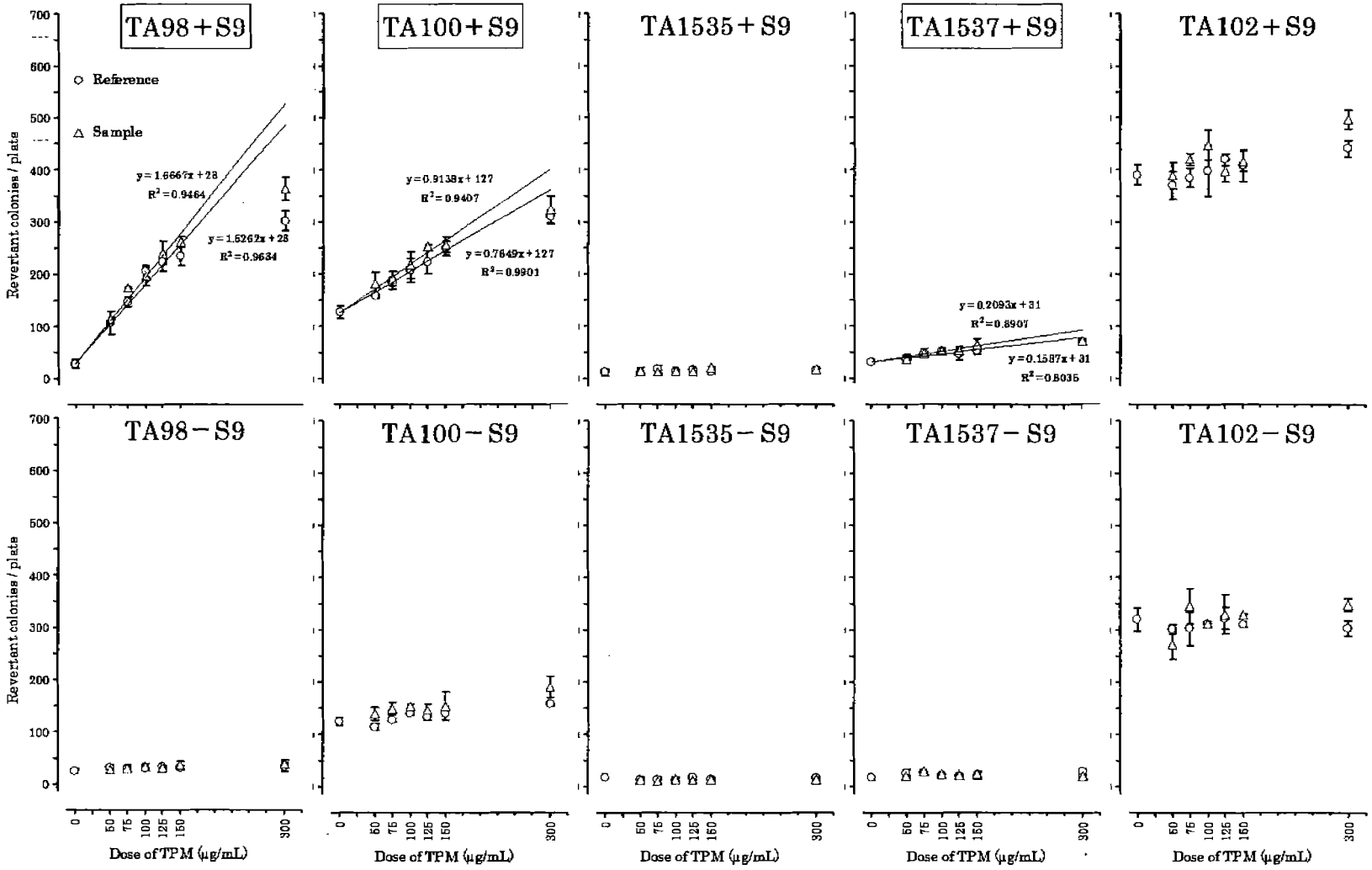
There were occasional statistically significant differences in hematology and clinical chemistry parameters from control values in groups exposed to smoke from test or reference cigarettes in both study 1 and study 2. These differences did not occur in a dose-response pattern and were well within ±2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



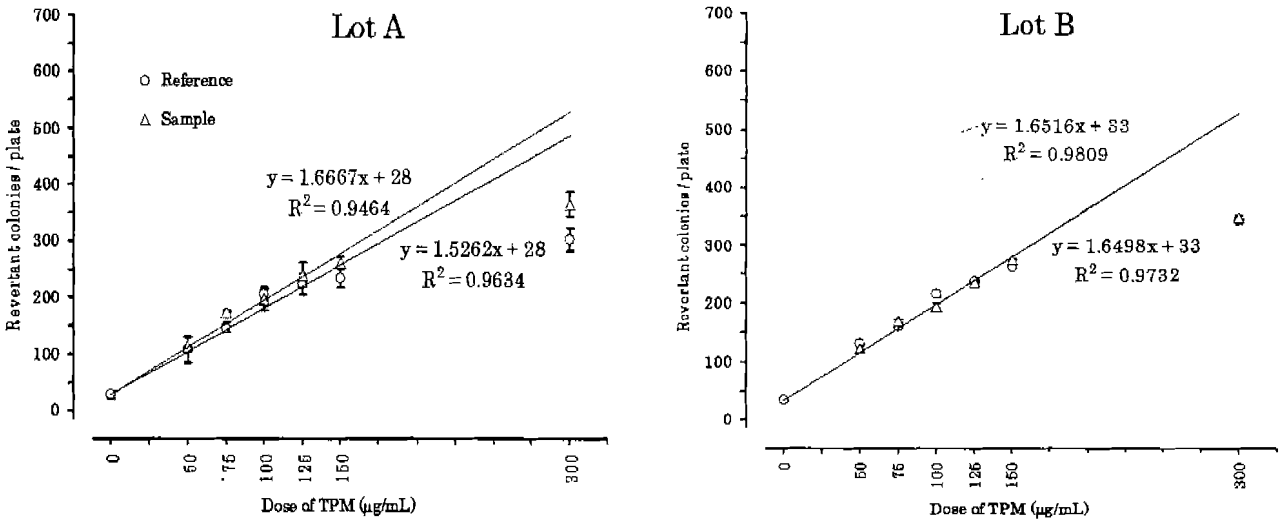
N=2. Only the first lot (Lot A) is indicated in this figure.
The second lot (Lot B) showed the same tendency as the first lot .

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

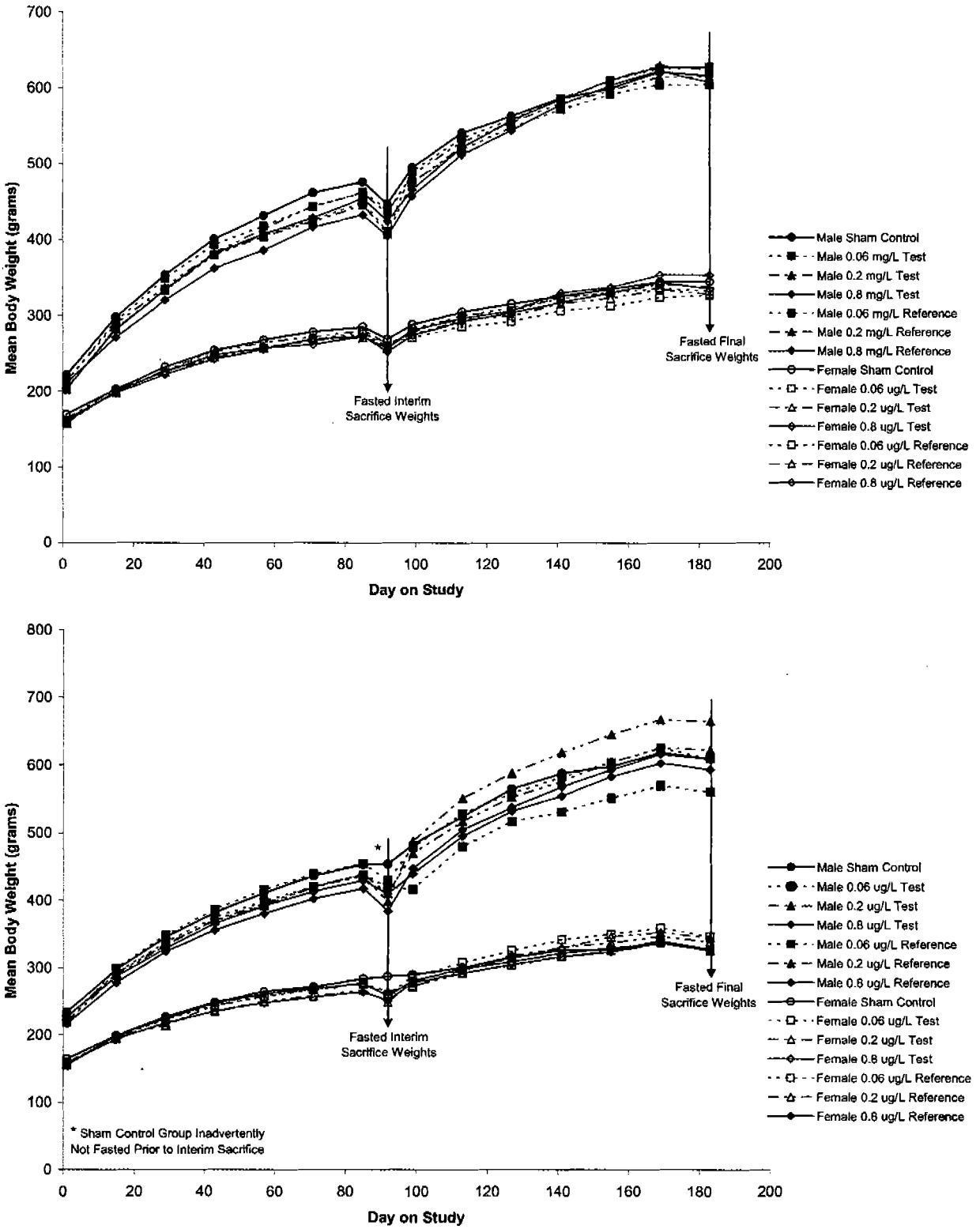


FIG. 5. Body weights, study 1 (top) and study 2 (bottom).

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 COMMISSION

Directorate-General for Health & Consumers

Scientific Committee on Consumer Safety

SCCS

OPINION
on
Fragrance allergens in cosmetic products

Scientific Committees
on consumer safety
on emerging and newly identified health risks
on health and environmental risks

The SCCS adopted this pre-consultation opinion at its 13th plenary meeting

of 13-14 December 2011

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

Dr. 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 Institute, 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, 13-14 December 2011

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 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. A separate request has already been made for hydroxycitronellal, isoeugenol and the content of peroxides in limonene.

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 occurs.

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 (4), see also chapter 4.2.2. 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 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. 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 (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 ingredients, confirming the relevance of positive patch tests to the Fragrance Mix 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, artificial sandalwood, 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. Epidemiology of fragrance allergy

4.2.1. 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 (32), are used for diagnosing contact allergy to fragrance 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 (33, 34). 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 (35).

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

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.

4.2.2. 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 (36)	Consecutive patients	2000	3790	6.9
Hungary (37)		1998-1999	3604	8.2 (7.3–9.1)§
Czech Republic (38)		1997-2001	12058	5.8 (5.4–6.2)§
Ljubljana, Slovenia (39)	Consecutive patients	1989-1998	6129	5.9 (5.3–6.5)§
Germany (40)	Consecutive IVDK patients	1996-2002	59298	11.3 (11.0–11.5)§
Germany (41)	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 (42)	Patients (fragrance allergy suspected)	04/2005-06/2007	295	5.8 (3.4–9.1)§
The Netherlands (43)	Consecutive patients	09/1998-04/1999	1825	10.6 (9.2–12.1)
The Netherlands (44)	Patients (cosmetic allergy suspected)	1994-1998	757	14.8 (12.3–17.5)§
Leuven, Belgium (45)	Consecutive patients	1990-2005	10128	9.1 (8.6–9.7)§
Coimbra, Portugal (46)	Consecutive patients	07/1989-06/1999	2600	10.9 (9.7–12.2)§
Sheffield, UK (47)	Consecutive patients	1994-1995	744	11.4 (9.2–13.9)§
St. John's, London, UK (48)	Consecutive patients	1980-2004	34072	7.7 (7.4–8.0)§
Copenhagen, Denmark (49)	Consecutive patients	1985-2007	16173	7.2 (6.8–7.6)§
ESSCA (50)	Consecutive patients	2002-2003	9663	7.1 (6.6–7.6)§
ESSCA (51)	Consecutive patients	2004	9941	7.6 (7.1–8.2)§
ESSCA (52)	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 (53)	Consecutive patients	04/2002–06/2003	422	9.7 (7.1–13.0)§
Lahore, Pakistan (54)	Dermatitis patients	2 years prior to 2002	350	7.7 (5.2–11.0)§
Manipal, India (55)	Dermatitis patients	1989-1998	1780	3.1 (2.3–4.0)§
Tel Aviv, Israel$(56)	Consecutive patients	1999-2000	943	8.5 (6.8–10.5)§
Tel Aviv, Israel (57)	Consecutive patients	1998-2004	2156	7.1 (6.1–8.3)§
Tehran, Iran (58)	Consecutive patients	2002-2004	250	4.0 (1.9–7.2)§
Ankara, Turkey (59)	Consecutive patients	1992-2004	1038	2.1 (1.3–3.2) §
Beijing, China (60)	Consecutive patients	2000-2003	378	15.9 (12.3–20.0)§
USA (Canada) (61)	Probably consecutive patients	2003	1603	5.9
NACDG 2009 (US and Canada) (62)	Consecutive patients	2005-2006	4439	11.5

Note: $ Possibly included in (57).

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 (40). A similar drop from 1999 to 2007 has been observed in female, but not male patients from Copenhagen (49). 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 (39).

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 (63). 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 (64). 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 (50). Subsequently, in the year 2004, the overall prevalence was 7.6%, i.e. largely unchanged (51). 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 (52).

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 (33)	Six clinical depts.	10/2002-06/2003	1701	2.9 (2.2–3.9)§
Germany (65)	IVDK patients	01/2005-12/2008	35633	4.9 (4.7–5.1)§
Groningen, Netherlands (42)	Patients (fragrance allergy suspected)	04/2005-06/2007	227	9.3 (5.8–13.8)§
Leuven, Belgium (45)	Consecutive patients	2005 only	335	2.1 (0.8–4.3)§
Denmark (66) 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 (67) and later included in the new perfume mixture, Fragrance Mix II (68), 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 (35) and detects relevant fragrance sensitisation which would otherwise have been missed (69). 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 (52). 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 (70)	Consecutive patients	04/2006-10/2008	816	0.9 (0.3–1.8)§
Spain (69)	Consecutive patients	10/2005-06/2008	852	0.8 (0.3–1.7)§
Germany (CH, AT) (71)	Consecutive patients	03/2000-02/2001	3245	1.9 (1.5–2.4)§
Germany (CH, AT) (72)	Consecutive patients	01/2003-12/2004	21325	2.4 (2.2–2.6)§

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI)
Germany (CH, AT) (65)	Consecutive patients	01/2005-12/2008	35582	2.3 (2.2–2.5)§
Belgium (45)	Consecutive patients	2002-2005	2901	2.1 (1.6–2.7)§
Denmark (66)	Consecutive patients	2005-2008	12302	2.4 (2.1–2.7)§
South Korea (53)	Consecutive patients	04/2002–06/2003	422	1.7 (0.6–3.4)§
USA, Canada (61)	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 (73). 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 (56) #	Consecutive patients	1999-2000	943	6.6 (5.1–8.4)§
South Korea (53)	Consecutive patients	04/2002 – 06/2003	422	7.3 (5.1–10.3)§
Tel Aviv, Israel (57)	Consecutive patients	1998-2004	2156	3.6 (2.9–4.5)§
Manipal, India (55)	Dermatitis patients	1989-1998	1780	1.0 (0.5 – 1.5) §
Tehran, Iran (58)	Consecutive patients	2002-2004	250	2.4 (0.9–5.2)§
Sevilla, Spain (74)	Consecutive patients	2002-2004	863	5.8 (4.3–7.6)§
Ankara, Turkey (59)	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 (38)	Consecutive patients	1997-2001	12058	7.3 (6.8–7.8)§
Copenhagen, Denmark (49)	Consecutive patients	1985-2007	16173	3.9 (3.6–4.2)§

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI)§
Sweden (36)	Consecutive patients	2000	3790	6.5
Nine European countries (50)	Consecutive patients	2002-2003	9672	6.1
Germany, three Swiss and one Austrian Dept. (41)	Consecutive patients	2005-2008	36919	8.0 (7.7–8.3)
Ten depts. From seven EU countries (63)	Consecutive patients	1996-2000	26210	6.0
USA (Canada) (61)	Probably consecutive patients	2003	1603	6.6
NACDG 2009 (62)	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 (75); virtually no .delta.-3-carene	Consecutive patients	1979-1983	4316	2.3 (1.9–2.8)§
Birmingham, UK (76)	Potters with occup. hand dermatitis	6 months; prior to 1996	24	14/4 pos. to "Indonesian turpentine"
Austria/Germany (IVDK) (77)	Consecutive patients	1992-1995	27658	0.47 (0.39–0.55)§
Austria/Germany (IVDK) (40)	Consecutive patients	1996-2002	59478	Annual prevalence 1.6 to 4.4%
Augsburg, Germany (78)	Population sample	1998	1141	1.2% (on population level!)
Europe (ESSCA) (50)	Consecutive patients	2002/03	3767	1.6%
Austria/Germany/ Switzerland (IVDK) (41)	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 (41,

65), 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).

4.2.3. 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 (79). 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 (80). 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 (81). A control group of 619 female patients with no eczema disease were also patch tested; 1.3% were positive to the Fragrance Mix (81). On the other hand, in a sample of 593 healthy Italian recruits, only three positive reactions (0.50%) to FM I were observed (82). Among Danish school children, 14-15 years of age, fragrance contact allergy was detected in 1.8% by patch testing with Fragrance Mix I (83). 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 (84). 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* (80, 85, 86). 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 (80, 85, 86).

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 (87) and adjusted multifactorial analyses (88). 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) (78), 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 (81)	Females without eczema	Not given	619	1.3
Italy (82)	Male recruits	Not given	593	0.50

Country (Ref.)	Population	Year(s)	No. tested	% positive (95% CI)
Denmark (80)	Population sample adults,15-69 years	1990-91	567	1.1
Denmark (83)	School children 12-16 years old	1995/96	717	1.8
Denmark (80, 85)	Population sample adults, 18-41 years	Jan-Nov 1998	414	2.7
Denmark (86)	Population sample adults, 18-69 years	June 2006–May 2008	3460	1.6
Norway (89)	Population sample adults,18-69 years. (Results reported in 2007)	1994 (90)	1236	1.8 (1.1–2.7)
Germany (78)	Subgroup of MONICA sample, age 25-74	1994/95	1141	11.4
USA (84)	Student nurses, females	1980	85	17.6*
Sweden (79)	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 (91)	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 (78)	Subgroup of MONICA sample, age 25-74	1994/95	*Myroxylon pereirae*	1141	2.4
Denmark (86)	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 (73).

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

4.3.1. 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 (92-95).

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.

4.3.2. 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 number of 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 (62)

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 ((62) 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 (64)

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 (68)).

Frosch 2002 (b) study (96)

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 (33)

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 (66)

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, 97, 98). Moreover, commercially available fragrance formulae and dilutions of individual fragrance allergens were potent elicitors of allergic contact dermatitis under simulated use conditions (10, 99, 100).

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 (101-103). 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 (104). These studies are discussed in more detail in chapter 11 on quantitative aspects. Other new studies are mentioned below:

IVDK "own perfumes" study (105)

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 ((105) 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 ((105) 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.

4.3.3. 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.

4.4. Socio-economic impact of contact allergy

4.4.1. Health related quality of life

Skin diseases in general are known to affect quality of life significantly (106); this also applies to eczema, where most studies concern atopic dermatitis and hand eczema patients (107, 108). Hand eczema has a poor prognosis and may affect the self-image, limit social activities and lead to occupational restrictions (108, 109). The quality of life in hand eczema patients with fragrance contact allergy is affected in a similar degree as patients with other contact allergies (110).

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 (111).

4.4.2. Occupational restrictions

Contact allergy is known to influence severity and prognosis of hand eczema (112, 113) including risk of sick leave (110). Fragrance contact allergy is mostly of a non-occupational origin (88) 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.

4.4.3. 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 (86). Further, as mentioned above, fragrance allergy may lead to sick leave (111). 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 (114). 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.

4.5. 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.

4.5.1. 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 4.4). Moreover, it is a common phenomenon and therefore a reduction of exposure to potential (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.

4.5.2. 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 (95) 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 (95) or guidelines (e.g. (115)); (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" (62), 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 4.3). 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 (116). 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 (111), most responded that they used the ingredient labelling (86.3%) and of those who used it, the majority (65.3%) found it helpful (111). 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.

4.6. 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 (117).

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) (118, 119). 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 (120). Secondary oxidation products such as aldehydes and epoxides can also be allergenic, thus further increasing the sensitisation potency of the autoxidation mixture (121). 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 (118, 122-127) (see chapter 8). This is also demonstrated for alpha-terpinene and citronellol (AT Karlberg, personal communication 2011). The oxidised fragrance terpenes limonene, linalool and linalyl acetate have been tested in consecutive dermatitis patients and give frequent allergic contact reactions (128-133). Details are given in Table 5-1

In contrast, the non-oxidised compounds rarely cause allergic reactions (41-43, 64, 67, 72, 96, 134-136), for details see Table 5-2. Not all oxidised fragrance substances are strong sensitisers, e.g. caryophyllene is readily oxidised but has a low sensitisation potency after autoxidation (137). This is supported by clinical studies showing oxidised caryophyllene to be a less frequent allergen compared to oxidised limonene and oxidised linalool (131).

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).

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%)	§ (128)
		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%	§ (129)
D-Limonene (ox.)	5989-27-5, 5989-54-8, 138-86-3	3	49/1812 (2.3%)	§ (132)
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%	§ (131)
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%)	§ (133)
		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%)	(138)
Linalyl acetate (ox.)	115-95-7	6	13/1217 (1.1%)	(139)

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	(134)
Limonene			3/2396 (0.1%)	§ (72)
DL-Limonene			11/1241 (0.88%)	§ (41)
Limonene			0/320	(42)
DL-Limonene			3/2396 (0.1%)	§ (72)
Linalool	78-70-6	30	0/179	(136)
		20	3/1825 (0.2%)	§ (43)
		10	2/320 (0.6%)	(42)
		10	4/792 (0.5%)	(135)
		5 and 1	0/100	(67)
Linalool, "stabilised" *		10	7/2401 (0.3%)	§ (72)
		10	2/985 (0.2%)	§ (41)
Linalyl acetate	115-95-7	1, 5	0/100	(67)
		10	4/1855 (0.2%)	§ (64)
beta-Caryophyllene	87-44-5	5	10/1606 (0.6%)	§ (96)

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

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 (128-133); see Table 5-3.

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	(128)*
	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	(130)*
	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	(132)*
	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	(131)*
	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	(133)*
	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.

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).

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 (139, 140). 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.

For prehaptens, the activation outside the body can be prevented to a certain extent. This is possible by measures during handling and storage of the ingredients and the final product to avoid air exposure and/or by the addition of suitable antioxidants. Prevention of autoxidation using antioxidants needs thorough investigation, as the autoxidation rate depends not only on the compound itself, but also its purity (141). Furthermore, it should be noted that most antioxidants exert their function by being activated instead of the compound that they protect, thus suggesting that they too could act as prehapten skin sensitisers. This is a risk to be considered given that antioxidants are now frequently used at increased concentrations in scented products due to a growing awareness of the problem of autoxidation.

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 (142), 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 (142). These enzymes are known to catalyse both activating and deactivating biotransformations (143), 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 (144-146), the allylic primary alcohols geraniol (119) cinnamyl alcohol (147-151), eugenol and isoeugenol (152).

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 (119, 147-151).

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 the use of isoeugenol in fragranced products needs to be indicated on the ingredients list, this important fragrance material may be replaced in fragrance formulations by derivatives with a similar scent. 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) (153). 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.

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 potent allergens 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 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 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 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.

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 (154-156). The categorisation of skin sensitisers according to sensitising potency has also been proposed (157). 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.

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" up to March 2010 (for the 26 "annex substances" from 1999 to October 2010), identifying all studies with fragrance substances.
2. PubMed search of CAS number 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.
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 classified as skin sensitisers (R 43).

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, by the local lymph node assay (LLNA) in mice, guinea pig maximisation test (GPMT) and Buehler test, to be presented in a structured format. In response, industry submitted first a poster (158) and later a report consisting of

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

LLNA protocol summaries on the 59 fragrance substances in the poster (159). 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 (160, 161) 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 (94, 95).
- 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.

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 (121, 162).

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 result in an animal study carried out according to accepted guidelines, providing unequivocal 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*)

[3] It should be noted that the SCCS considers such tests unethical (163).

or

- A positive result in at least one 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
- A positive result in at least one 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*).

6.4. 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 at the beginning of this chapter, were grossly categorised according to the number of patients tested and the number of patients reacting positively, based on the publications considered. 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.

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) ROSE KETONE-4	23696-85-7	+ (r.t.)
alpha-DAMASCONE (TMCHB)[#]	43052-87-5 / 23726-94-5	++
cis-beta-DAMASCONE [#]	23726-92-3	+

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

INCI name (or, if none exists, perfuming name according to CosIng)	**CAS number**	**Comment: see text**
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	++
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	+

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

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment: see text
TETRAMETHYL ACETYLOCTAHYDRONAPHTHALENES	54464-57-2 / 54464-59-4 / 68155-66-8 / 68155-67-9	+
TRIMETHYL-BENZENEPROPANOL (Majantol)	103694-68-4	++
VANILLIN	121-33-5	++

In Table 7-2, those substances are listed 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, regarding 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	(164)
CARVACROL	499-75-2	2 of 28 patients	(Meynadier, after (165))
CUMINALDEHYDE	122-03-2	3 of 179 patients positive	(136)
CYCLOHEXYL ACETATE	622-45-7	0.5% positive of 218 selected patients	(166)
CYCLOPENTADECANONE	502-72-7	3 of 178 patients positive	(164)
trans-trans-delta-DAMASCONE	71048-82-3	1 positive HRIPT (2/15 with 1%)	(167)
2,3-DIHYDRO-2,2,6-TRIMETHYLBENZALDEHYDE	116-26-7	1 positive HRIPT (5 of 53)	(168).
DIMETHYLTETRAHYDRO BENZALDEHYDE	68737-61-1	2.3% positive reactions isomer mixture in 178 patients	(164)
ETHYLENE DODECANEDIOATE	54982-83-1	2 / 218 positive PT reactions	(166)
ETHYL VANILLIN	121-32-4	1 occupational case	(169)
HELIOTROPINE	120-57-0	6 / 1606 consecutive patients positive	(96)
HYDROXYCITRONELLOL	107-74-4	6.0% positive PT reactions in 218 patients	(166).
ISOAMYL SALICYLATE	87-20-7	1 positive in 179 patients, possibly "excited back syndrome"	(136). (67)

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment	Ref.
		0 / 95 in another study with <= 1/10 of above test conc.	
ISOLONGIFOLENEKETONE	33407-62-4	1 / 178 patients	(164)
METHOXYCITRONELLAL	3613-30-7	Positive PT data of unknown validity by Nakayama et al. in 22/137 patients.	(170)
METHOXYTRIMETHYLHEPTANOL	41890-92-0	0.9% positive PT	(166)
METHYL p-ANISATE	121-98-2	1 / 182 patients positive	(171)
METHYL CINNAMATE	103-26-4	6 / 142 patients positive	(172)
METHYL DIHYDROJASMONATE	24851-98-7	3 / 1606 patients positive 0 / 100	(96) (67)
METHYLIONANTHEME	55599-63-8	1 case	(173)
5-METHYL-alpha-IONONE	79-69-6	5 / 1606	(96)
METHYL OCTINE CARBONATE	111-80-8	1 case	(174)
MYRCENE	123-35-3	1 / 1511 positive to oxidized myrcene	(131)
MYRTENOL	515-00-4	2 HRIPTs with 1 pos. each	(175)
NEROL	106-25-2	6.0% positive	(166)
Nerolidol (isomer not specified)	7212-44-4	Few, unconfirmed pos. cases according to RIFM review	(176)
NOPYL ACETATE	128-51-8	2 / 179 positive, possibly "excited back syndrome"	(136)
PHENETHYL ALCOHOL	60-12-8	1 / 179; 0 / 100	(136) (67)
PHENYLACETALDEHYDE	122-78-1	1.1% of 182 positive. 1 case	(171) (177).
PHENYLPROPANOL	122-97-4	2 / 218	(166).
PHYTOL	150-86-7	1 case in human max. test	(178)
RHODINOL	6812-78-8	Several pos. HRIPTs, clinical data of uncertain validity	(179)
trans-ROSE KETONE-5	39872-57-6	2 / 22 pos. HRIPT	(180)

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	(67)
AMYLCYCLOPENTANONE	4819-67-4	0 / 178	(164)
BENZYL ACETATE	140-11-4	0 / 100 consecutive patients in 1 centre observed	(67)
2-TERT-BUTYLCYCLOHEXYL ACETATE	88-41-5	0 / 313 consecutive patients in 2 centres	(67)
4-tert.-Butylcyclohexyl acetate	32210-23-4	0 / 107 consecutive patients in 1 centre observed	(67)
6-ETHYLIDENEOCTAHYDRO-5,8-METHANO-2H-BENZO-1-PYRAN	93939-86-7	0 / 178	(164)
3a,4,5,6,7,7a-HEXAHYDRO-4,7-METHANO-1H-INDEN-5(OR 6)-YL ACETATE	54830-99-8	0 / 313 consecutive patients in 2 centres	(67)
HEXYL SALICYLATE	6259-76-3	0 / 218 "top 100" substance and classified as R43	(166)
HIBISCOLIDE	6707-60-4	0 / 178	(164)
alpha-IONONE	127-41-3	0 / 205	(67)
beta-IONONE	79-77-6	0 / 205 "top 100" substance	(67)
ISOBORNYL ACETATE	125-12-2	0 / 107 "top 100" substance	(67)
METHYL ANTHRANILATE	134-20-3	0 / 91 "top 100" substance	(181)
METHYL IONONE (mixture of isomers)	1335-46-2	0 / 100 "top 100" substance	(67)
OXALIDE	1725-01-5	0 / 178	(164)

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Results / Comment	Ref.
TERPINEOL ACETATE (Isomer mixture)	8007-35-0	0 / 106 "top 100" substance	(67)
alpha-TERPINYL ACETATE	80-26-2	0 / 179	(136)
TRIMETHYL-PROPYLCYCLOHEXANEPROPANOL	70788-30-6	0 / 178	(164)

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

[#] Annex III, part 1

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number
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
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 (182).

The three main methods used to concentrate plant fragrance substances; 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 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) 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 (182).

Regarding clinical data in terms of contact allergy to fragrance ingredients, the main focus of case reports or clinical studies on 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, physiotherapists (183, 184), aromatherapists (185-189), beauticians performing massages (190). For further details, e.g. PT results with various essential oils, see the original case reports.

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.

INCI name (or, if none exists, perfuming name according to CosIng)	**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 *LICHEN EXTRACT* [4] *(Tree moss)*	*90028-67-4*	+++
EVERNIA PRUNASTRI*(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*	++

[#] 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
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;	++++
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 (*Lippia citriodora* Kunth.) #	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)	CAS number	Comment	Ref.
ACORUS CALAMUS *ROOT OIL*	*84775-39-3*	n=7 pos. reactions to "calamus"	(191)
CEDRUS DEODARA *WOOD OIL*	*91771-47-0*	Rudzki 1976/1986 found 3 / 3 positive reactions	(191, 192).
CITRUS AURANTIUM AMARA *LEAF OIL*	*72968-50-4*	Several cases in 2 series from 1 centre	(191, 192).
CITRUS TANGERINA …	*223748-44-5*	1 case	(193)
CYMBOPOGON NARDUS / WINTERIANUS *HERB OIL*	*89998-15-2; 91771-61-8*	Several cases in 2 series from 1 centre	(191, 192).
ILLICIUM VERUM *FRUIT OIL*	*84650-59-9*	Cases of active	(194)

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment	Ref.
		sensitisation; 34% consecutive patients pos. to 1%	
LAVANDULA SPICA	*97722-12-8*	Several cases in 2 series from 1 centre	(191, 192).
LITSEA CUBEBA	*90063-59-5*	Several cases in 2 series from 1 centre	(191, 192).
PELARGONIUM ROSEUM	*90082-55-6*	2.1% pos. of 1483 patients	(195).
ROSMARINUS OFFICINALIS	*84604-14-8*	3 cases in 2 series from 1 centre	(191, 192).
SALVIA spp.	*Diverse*	Several cases in 2 series from 1 centre	(191, 192).
TAGETES PATULA	*91722-29-1*	1 case (aromatherapist)	(185)
THYMUS spp.	*84929-51-1*	4 / 84 pos	(191)
VETIVERIA ZIZANOIDES	*8016-96-4; 84238-29-9*	1 / 200 and 9 / 86 pos.	(191, 192)

The last 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)	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% (196).
MENTHA ARVENSIS	68917-18-0	high volume, classified as R43
OCIMUM BASILICUM	*84775-71-3*	Pos. LLNA study by RIFM: EC3 value < 2.5% (196).
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 4.2.1, 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[5] and the local lymph node assay (LLNA)[6]. These methods are used in hazard identification and risk assessment for regulatory purposes under REACH[7]. 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[8], 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[9]) "H317: May cause an allergic skin reaction".

As yet, there is no validated *in vitro* test method accepted 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 (157, 197). 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 (157): 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 (157, 197).

The potency categorisation of substances based on the LLNA is applied by the SCCP in risk assessment of cosmetic ingredients, particularly hair dye substances (198).

8.1.1. LLNA data

The SCCS requested the International Fragrance Association (IFRA) to submit data on animal tests performed with fragrance substances, by the local lymph node assay (LLNA) in mice, the guinea pig maximisation test (GPMT) and the Buehler test, and presented in a structured format. In response, IFRA submitted first a poster (158) and later a report

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

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

[7] 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).

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

[9] Regulation No. 1272/2008.

consisting of LLNA protocol summaries on the 59 fragrance substances in the poster (159). 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 (159). EC3 values for some additional fragrance substances in two published reviews (160, 161) 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 (159)) and in published reviews by Gerberick et al. 2005 (160) and Kern et al. 2010 (161), 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	(159, 161)
Cinnamal	104-55-2	0.2	0.015	(159)
Methyl 2-octynoate	111-12-6	<0.5	<0.032	(159, 161)
Isoeugenol	97-54-1	0.54	0.033	(159)
Citral	5392-40-5	1.2	0.079	(159)
2-Hexylidene cyclopentanone	17373-89-6	2.4	0.14	(159)
Methyl octine carbonate	111-80-8	2.5	0.15	(159)
Peru balsam absolute	8007-00-9	2.5	n/a	(159)
trans-2-Hexenal	6728-26-3	2.6	0.26	(159)
Benzyl Salicylate	118-58-1	2.9	0.23	(159, 161)
Butylphenyl methylpropional (BMHCA)	80-54-6	2.9	0.14	(159)
Phenylacetaldehyde	122-78-1	3	0.25	(159, 160)
Allyl phenoxyacetate	7493-74-5	3.1	0.16	(159)
Benzylideneacetone	122-57-6	3.7	0.25	(160)
3-Propylidenephthalide	17369-59-4	3.7	0.21	(159, 160)
Evernia prunastri extract oak moss	90028-68-5	3.9	n/a	(159)
Balsam oil, Peru *(Myroxylon pereirae Klotzsch)*	8007-00-9	4	n/a	(159)
Farnesol	4602-84-0	4.1	0.18	(159)
p-t-Butyl-dihydrocinnamaldehyde	18127-01-0	4.3	0.23	(159)
α-Methyl cinnamic aldehyde	101-39-3	4.5	0.31	(159, 160)
Eugenol	97-53-0	5.3	0.32	(159)
Hexyl cinnamal	101-86-0	5.3	0.25	(159)
Dihydrocoumarin	119-84-6	5.6	0.38	(160)

Substance	CAS no.	EC3 value		Reference
		%	M	
Geraniol	106-24-1	5.6	0.36	(159)
Carvone	6485-40-1	5.7	0.38	(159)
Diethyl maleate	141-05-9	5.8	0.34	(160)
2-Methoxy-4-methylphenol	93-51-6	5.8	0.42	(159, 160)
Anise alcohol	105-13-5	5.9	0.43	(159, 161)
Jasmine absolute *(Grandiflorum)*	8022-96-6	5.9	N/a	(159)
Dibenzyl ether	103-50-4	6.3	0.32	(159)
Cananga odorata leaf/flower oil ylang ylang "extra"	8006-81-3	6.8	N/a	(159)
Isocyclocitral	1335-66-6	7.3	0.48	(159)
2,3-Dihydro-2,2,6-trimethylbenzaldehyde	116-26-7	7.5	0.50	(160)
Amyl cinnamal	122-40-7	7.6	0.38	(159)
Perillaldehyde p-Mentha-1,8-dien-7-al	2111-75-3	8.1	0.54	(159, 160)
p-Isobutyl-α-methyl hydrocinnamaldehyde	6658-48-6	9.5	0.46	(159)
d-Limonene*	5989-27-5	<10	<0.73	(159)
Methylundecanal	110-41-8	10	0.54	(160)
Acetylcedrene	32388-55-9	13.9	0.57	(161)
Methylenedioxyphenyl methylpropanal	1205-17-0	16.4	0.85	(159, 161)
Benzyl benzoate	120-51-4	17	0.80	(160)
Hydroxyisohexyl 3-cyclohexene carboxaldehyde	31906-04-4	17.1	0.81	(159, 160)
Benzyl cinnamate	103-41-3	18.4	0.77	(159, 161)
Hydroxycitronellal	107-75-5	19.3	1.12	(159)
Cinnamyl alcohol	104-54-1	21	1.57	(160)
α-iso-Methylionone	127-51-5	21.8	1.06	(159, 161)
Cyklamen aldehyde	103-95-7	22	1.64	(160)
4-Methoxy-α-methyl benzenpropanal	5462-06-6	23.6	1.32	(159)
Amyl cinnamyl alcohol	101-85-9	~25	~1.22	(159, 161)
Tetramethyl acetyloctahydronaphthalenes (OTNE)	54464-57-2	25.1	1.07	(159)
Ethyl acrylate	140-88-5	28	2.8	(160)
Linalool*	78-70-6	30	1.94	(160)
Trimethylbenzenepropanol Majantol	103694-68-4	30	~1.68	(159)
Jasminum Sambac Flower CERA/Extract/Water	91770-14-8	35.4	N/a	(159)
Citronellol	106-22-9	43.5	2.78	(159, 161)
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	(159)

Substance	CAS no.	EC3 value		Reference
		%	M	
Camellia sinensis leaf tea leaf absolute	84650-60-2	>5	N/a	(159)
Cinnamyl nitrile	1885-38-7	>10	>0.77	(159)
Menthadiene-7-methyl formate	68683-20-5	>10	>0.51	(159)
Evernia furfuracea extract tree moss absolute	90028-67-4	>20	N/a	(159)
Isocyclogeraniol	68527-77-5	>25	>1.62	(159)
1-Octen-3-yl acetate	2442-10-6	>30	>1.76	(159)
Benzyl alcohol	100-51-6	>50	>4.62	(159)
Coumarin	91-64-5	>50	>3.42	(159)
Vanillin	121-33-5	>50	>3.3	(159)
No EC3 value calculated				
Benzaldehyde	100-52-7	-		(160)

Notes: * Material with low levels of oxidation according to (159)
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	(199)
D-Limonene (pure)	5989-27-5	25, 50, 100	30	
Linalool (ox. 10 w)	78-70-6	5, 10, 25	9.4	(126)
Linalool (ox. 45 w)	78-70-6	2.5, 10, 25	4.8	
Linalool (pure)	78-70-6	25, 50, 100	46.2	

Substance	CAS no.	Doses % (w/v) vehicle: A:OO 4:1*	EC3 value (% w/v)	Reference
Linalyl acetate (ox. 10 w)	115-95-7	0.5, 10, 40	3.6	(127)
Linalyl acetate (pure)	115-95-7	10, 30, 100	25	
Geraniol (ox. 10 w)	106-24-1	1, 3, 6, 10, 20	4.4	(118)
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 (158) and the report by IFRA (159), 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 (157, 197), and these have been introduced into Table 8-1. The EC3 values in the reviews by Gerberick et al. and Kern et al. (160, 161) 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 (159) 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 (159). 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 (159) 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 (159-161) as assessed by EC3 values in the LLNA, is shown in Figure 8-1 and Table 8-3.

The median EC3 value of 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.

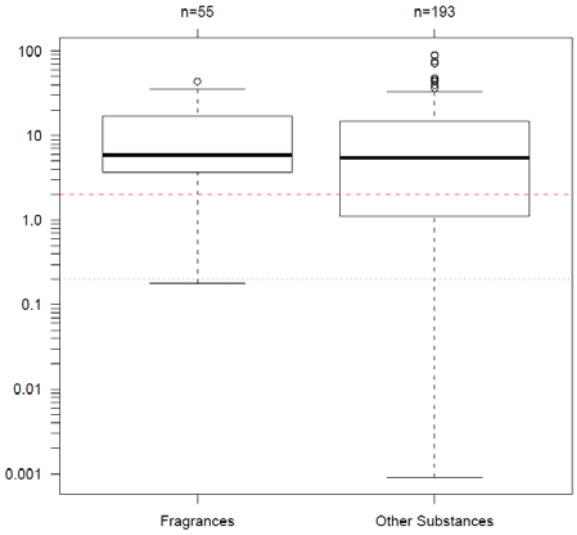


Figure 8-1: The distribution of fragrance chemicals and a variety of other chemicals (e.g. biocides, dyes, plastic materials), taken from the three references (159-161), are depicted as boxplots on a logarithmic scale. The bottom of the box denotes the 1st quartile (25% percentile), the thick line in 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 (159-161). The EC3 value intervals for potency categorisation (157, 197) were used for comparison of fragrances substances vs other substances.

EC3 value interval	Fragrance substances		Other substances	
	no.	%	no.	%
≤ 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 (159) and assessed in this opinion were generated exclusively by LLNA. Other guideline methods are, however, also available.
- The vast majority of the submitted (159) and additional (160, 161) fragrance substances tested by the LLNA are skin sensitisers.
- Several studies in the IFRA report (159) 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. 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. 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 (200). 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. See also chapter 5.

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 (201, 202). 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 (203, 204).

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 this section summarised in Table 9-3 and Table 9-4 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 (205), whereas the LLNA potency of DNCB analogues (the S_NAr domain) is well modelled by reactivity alone (206).

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, see chapter 5), 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. We can 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. (121).

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 (Table 9-1 to Table 9-4) based on the prediction made for the actual substance. The following SAR assessments have been used:

- Predicted sensitiser; structural alerts (Table 9-1).
- Possible sensitiser; structural alerts (Table 9-2).
- Predicted non-sensitiser (NS); no obvious structural alerts (Table 9-3).
- Not predictable due to insufficient/conflicting data (Table 9-4).

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 acetate	54830-99-8	Possible prehapten
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

Substance (INCI) name	CAS number	Structural alerts
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	
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	

Substance (INCI) name	CAS number	Structural alerts
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.

- SAR prediction is a useful tool for estimation of the sensitisation potential of those compounds that lack human and animal data as the skin sensitisation potential is closely connected to chemical reactivity.
- 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

- 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.

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 (68, 207-209). Cosmetics based on natural ingredients may contain fragrance allergens at a higher concentration than other cosmetic products (210). 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 perfume oil, compared to up to 30% perfume oils in EDT/EDP (211). The perfume oils are mixtures of 20 to over 200 synthetic fragrance chemicals or natural fragrance materials (essential oils), selected from over 3,000 fragrance materials (211). Perfume oil of the same composition is used in different concentrations in the formulation of various cosmetic products within a brand of cosmetics. 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, antioxidants, and skin penetration enhancers (for example isopropyl myristate), etc. (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, (212)) and (Table 10-2, (213)) and on consumer products including cosmetics (Table 10-3, (214); Table 10-4, (114); and Figure 10-1, (104)). 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 (215).

Table 10-1: Presence in children's cosmetics of the 26 fragrance substances that are required to be labelled in cosmetics (212).

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 (213)**			**70 products investigated in 1998 (216)**	
		% Products labelled to contain the fragrance	**Content in 23 selected products**		**Content in all 70 products**	
INCI name	**CAS number**		**% 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 (213)			70 products investigated in 1998 (216)	
		% Products labelled to contain the fragrance	Content in 23 selected products		Content in all 70 products	
INCI name	CAS number		% Products found to contain the fragrance	Range(ppm)	% Products found to contain the fragrance	Range (ppm)
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 (214).

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* (114).

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.

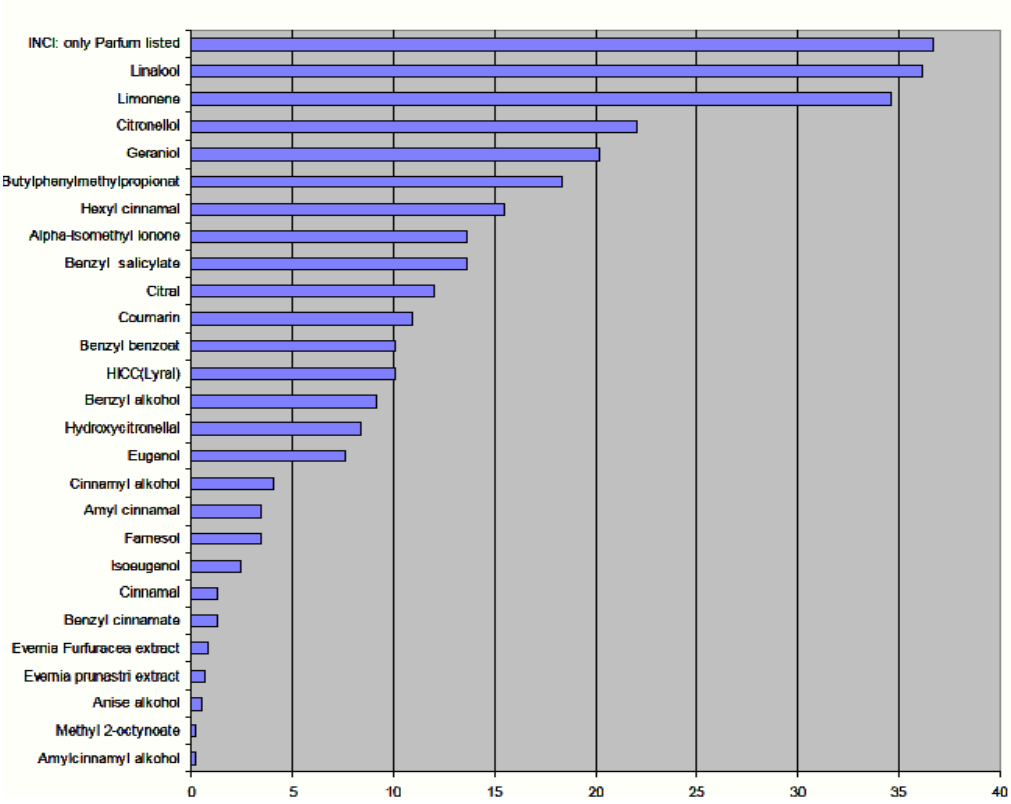


Figure 10-1: Frequency of occurrence in 3,000 consumer products of the 26 fragrance allergens that are required to be labelled in cosmetics and detergents (CVUA Karlsruhe, Germany, 2006/2007), according to (104).

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, 153, 217-219), deodorants (213, 216), children's cosmetics and cosmetic toys (114, 212, 220), products marketed as natural cosmetics (210) and in cosmetics used by patients with contact allergy to fragranced products (33, 68). 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), (104, 114, 214, 221), 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, 217). 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 (217)**		
	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. (213, 216). 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 (222, 223). The EC Scientific Committee on Consumer Products (SCCP) recommended that atranol and chloroatranol should not be present in cosmetic products (224). Two other commonly used fragrance chemicals, isoeugenol (225) and hydroxyisohexyl-3-cyclohexene carboxyaldehyde (HICC) (68), 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 (218). 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 (226). 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, (218, 219). 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 (227). 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 (220). It has been stated that children may become sensitised to fragrance chemicals used by their mothers (228).

Clothing

Washed fabrics have been reported to contain fragrances (229). 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 (230).

Cleaning agents and other household products

Contact dermatitis from geraniol in washing-up liquid has been reported (231). Terpenes are used as solvents and cleansing agents (e.g. limonene) (232) and have been reported as cause of hand dermatitits (233, 234). In an analysis of 59 household products the most common fragrance allergens were limonene (78%), linalool (61%) and citronellol (47%) (235). 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 (236). 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) (214).

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 (237).

Food

Food causing cheilitis or bullous stomatitis (e.g. due to cinnamal (238)) or lichen planus-like lesions (e.g. due to cinnamal (239)) or contact gingivitis (e.g. due to eugenol (240)) has been reported. Moreover, food containing fragrance allergens, e.g. citrus oil terpenes (241) 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 (88). 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 (87, 88, 242-244). Occupational exposure and occupational ACD to fragrances have been described in perfume bottlers (245). Industrial

use of a powder masking the vinyl smell of car seats, containing cinnamal, causing occupational ACD has been reported (244).

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".

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
Margosa ext.	283-644-7	84696-25-3	18, 19
Pine ext.	304-455-9	94266-48-5	10
Chrysanthemum vulgare	310-127-6	natural oil	Not given
Chrysanthemum cinerariaefolium, ext.	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, 45). 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 (246). 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 (247). 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 (248). A cumulative effect of exposures occurs so that repeating exposures cause elicitation in more individuals (249).

Patients appear to become sensitised to fragrances primarily from deodorants and perfumes and to a lesser extent from other cosmetic types (72). Allergic contact dermatitis may develop where a perfume has been applied (behind ears, neck, upper chest, antecubital fossae, wrists and the axillae bilaterally (250). 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 (251, 252), 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 (88). 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 (253)). 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 (88) 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 (87). This association is presumably the result of life long exposures and cumulative risk.

In a similar analysis of *Myroxylon pereirae* resin, published in 2002 (254): (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 (254). 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 (255). It seems that not only the dose per unit area of allergen, but also the number of exposures, i.e. the accumulated dose, is of importance for the risk of induction of contact allergy (256). 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 (257, 258) 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 4.2.2 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 (259). 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 (260) 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, 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 much better reflect actual exposure 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) (261), 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 (261). 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

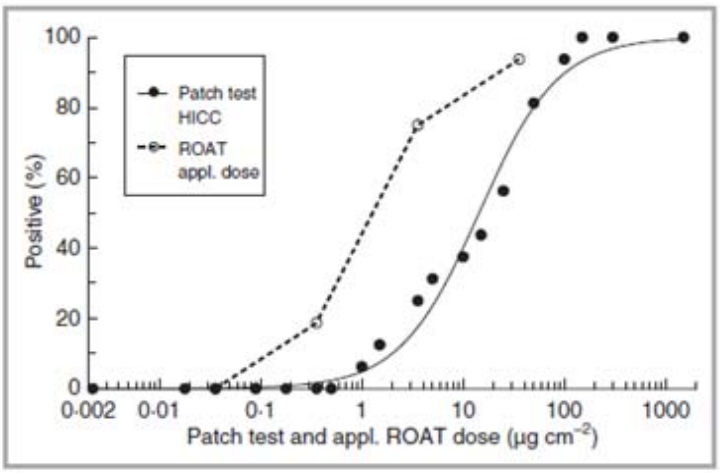


Figure 11-1: The fitted dose-response curve for patch test (solid line) is seen to be displaced to the right compared to the observed response from repeated open applications of the same allergen (HICC). It means that a smaller dose per application is needed to elicit a response than by one single occluded 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 (261). 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 (262):

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

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 (263). 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) (95-97, 243). 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	(264)	(102)		(103)	(101)

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	(223)
In ethanol 100% positive	0.125 µg/cm^2	observed	(223)
PATCH TEST			
ED10%	0.0004 µg/cm^2	estimated	(223)
ED50%	0.0045 µg/cm^2	estimated	(223)
Cinnamal			

ROAT			
In ethanol no effect	0.02%	observed	(100)
In ethanol 44 % positive	0.1%	observed	(100)
In ethanol 72 % positive	0.8%	observed	(100)
Deodorant matrix 11% positive	0.26 µg/cm^2 (0.01%)	observed	(102)
Deodorant matrix 41% positive	0.84 µg/cm^2 (0.032%)	observed	(102)
Deodorant matrix 82% positive	2.63 µg/cm^2 (0.1%)	observed	(102)
PATCH TEST			
ED50%	96 µg/cm^2	estimated	(100)
No effect level	0.4 µg/cm^2 (0.01%)	observed	(100)
No effect level	NG (0.002%)	observed	(102)
HICC			
ROAT			
In a cream base ED10%	4.9 µg/cm^2	interpolated	(104)
In a perfume (ethanol) ED10%	1.2 µg/cm^2	interpolated	(104)
In ethanol 61% positive	15.3 µg/cm^2 (3.4-22.2)	observed	(209)
In ethanol 89% positive	126.2 µg/cm^2 (40.5-226.2)	observed	(209)
In ethanol/water no response	0.0357 µg/cm^2	observed	(248)
In ethanol/water ED10%	0.064 µg/cm^2	estimated	(248)
In deodorant matrix between 64% to 100% positive	0.79 µg/cm^2 (median)	observed	(101)
PATCH TEST			
ED10% (95% CI)	0.662 µg/cm^2 (0.052-2.35)	estimated	(248)
ED10%	0.75 µg/cm^2	estimated	(101)
ED10%	0.9 µg/cm^2 29 (7-69) ppm	estimated	(209)
ED50% (95% CI)	11.1 µg/cm^2 (3.41- 33.1)	estimated	(248)
ED50% (95% CI)	18.3 µg/cm^2 (3.41- 33.1)	estimated	(101)
ED50% (95% CI)	20 µg/cm^2 662 (350-1250) ppm	estimated	(209)
No effect level	<0.0022 µg/cm^2	observed	(248)
Hydroxycitronellal			
ROAT			
Deodorant matrix 57 % positive	0.94 µg/cm^2 (0.032%)	observed	(103)
Deodorant matrix 71 % positive	2.94 µg/cm^2 (0.1%)	observed	(103)
Deodorant matrix 100 % positive	9.40 µg/cm^2 (0.32%)	observed	(103)
PATCH TEST			
No effect level	<0.00012 % (=0.036 µg/cm^2)* (*calculated)	observed	(103)
Isoeugenol			

ROAT			
in ethanol 63% positive	5.6 µg/cm^2	observed	(99)
in ethanol 42% positive	2.2 µg/cm^2	observed	(249)
in ethanol 67% positive	9.0 µg/cm^2	observed	(249)
Deodorant matrix 23 % positive	0.167 µg/cm^2	observed	(264)
Deodorant matrix 69 % positive	0.53 µg/cm^2	observed	(264)
Deodorant matrix 77 % positive	1.67 µg/cm^2	observed	(264)
PATCH TEST			
ED50% (in petrolatum)	32 µg/cm^2	estimated	(99)
No effect (in ethanol)	<0.0005% (0.15 µg/cm^2)	observed	(249)
No effect (in petrolatum)	<0.4 µg/cm^2	observed	(99)

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: (265)). 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 (265) and guinea pig QSAR evidence (266). 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 (265).
- Exposure doses and exposure areas from SCCS notes of guidance 7th revision (267) [Tables 2 and 3] and Technical dossier Quantitative Risk Assessment from RIFM (259).

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 (265).

ED10 patch test values from each of the 16 selected studies with 95 % confidence intervals with the allergens chromium (268), MCI/MI (Kathon ™ CG) (269), nickel (270), methyldibromo glutaronitrile (MDBGN) (271), hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) (101, 209, 248), isoeugenol (249, 264) and formaldehyde (272). 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
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

Study	Number of patients	ED_{10} (µg/cm^2)	95 % interval
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.

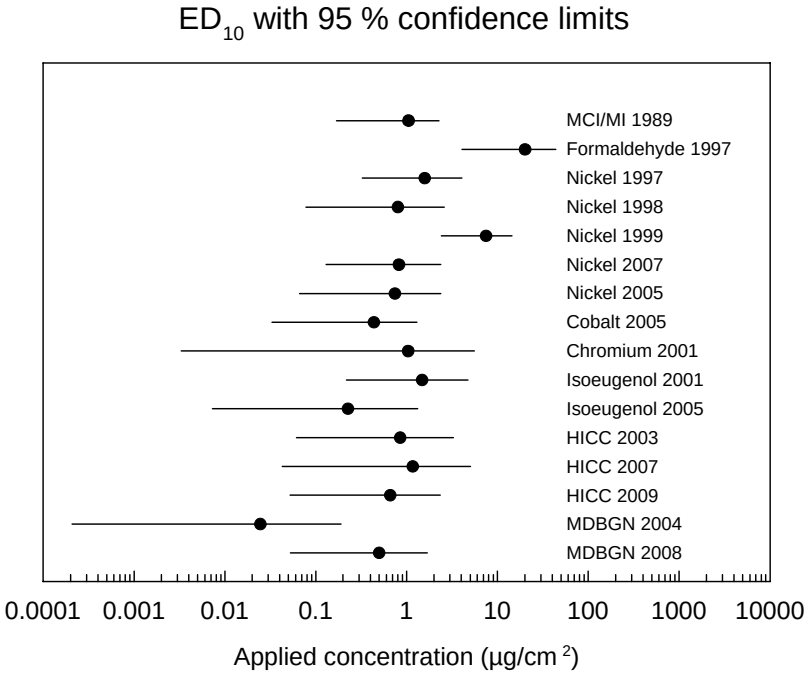


Figure 11-2: The threshold data with 95% confidence intervals from Table 11-6 presented graphically, after (265).

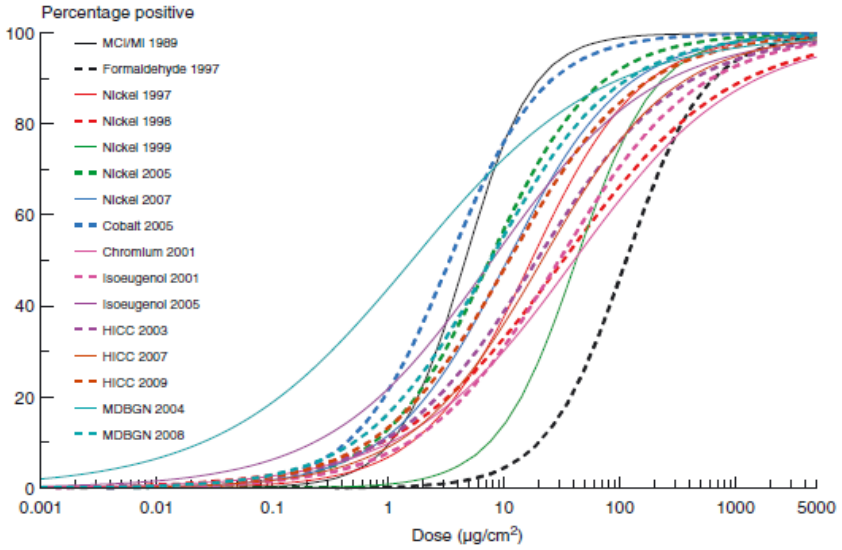


Fig. 1. Logistic dose–response curve for 16 patch test elicitation dose–response studies with methylchloroisothiazolinone/methyl isothiazolinone (MCI/MI) (8), formaldehyde (9), nickel (10–14), cobalt (14), chromium (15), isoeugenol (16, 17), hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) (18–20), and methyldibromo glutaronitrile (MDBGN) (21, 22).

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 (265).

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 (265).

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 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 (273) and in a reduction in morbidity, i.e. elicitation (274). Another example is restriction of chromium VI in cement (275).

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 (224). 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 (276)) (for more studies see chapter 4.2.2); in 70% of the cases the reaction was of current relevance, i.e. causing disease (66). 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 (104). In a Danish study 69% of 14 HICC allergic individuals developed allergic contact dermatitis from use of cosmetics containing HICC in realistic amounts (101).

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 (277). 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" (278). An overview of the IFRA restrictions is given in the table below.

Table 11-7: Restriction for HICC independent of the QRA according to (278).

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.

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 three 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.

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 (159) 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 (279).

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 (280) 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 fragrances 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.

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 (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-	+ (r.t.)

INCI name (or, if none exists, perfuming name according to CosIng)	**CAS number**	**Human evidence: see text**
	8	
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-CYCLOPENTENYL)PENT-4-EN-2-OL	67801-20-1	++ (r.t.)

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text
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 LICHEN EXTRACT*	**90028-67-4**	**+++**
EVERNIA PRUNASTRI*	**90028-68-5**	**+++**
JASMINUM GRANDIFLORUM / OFFICINALE	**84776-64-7; 90045-94-6; 8022-96-6**	**+++**
JUNIPERUS VIRGINIANA	8000-27-9; 85085-41-2	++

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text
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	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 (*Lippia citriodora* Kunth.)	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
Cinnamyl nitrile	1885-38-7	none	> 10
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
Isocyclocitral	1335-66-6	none	7.3

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)
Isocyclogeraniol	68527-77-5	none	> 25
α-Methyl cinnamic aldehyde	101-39-3	none	4.5
METHYLENEDIOXYPHENYL METHYLPROPANAL	1205-17-0	none	16.4
6-Methyl-3,5-heptadien-2-one	1604-28-0	none	> 5
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
1-Octen-3-yl acetate	2442-10-6	none	> 30
Perillaldehyde p-Mentha-1,8-dien-7-al	2111-75-3	none	8.1
PHENYLACETALDEHYDE	122-78-1	limited	3
Natural extracts			
Camellia sinensis leaf *Tea Leaf Absolute*	84650-60-2	none	> 5
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	+
CUMINALDEHYDE	122-03-2	limited	none	+
CYCLOPENTADECANONE	502-72-7	limited	none	+
trans-trans-delta-DAMASCONE	71048-82-3	limited	none	+
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	+
METHOXYCITRONELLAL	3613-30-7	limited	none	+
METHYL CINNAMATE	103-26-4	limited	none	++
METHYL EUGENOL	93-15-2	limited	none	++
METHYLIONANTHEME	55599-63-8	limited	none	+
5-METHYL-alpha-IONONE	79-69-6	limited	none	+
MYRCENE	123-35-3	limited	none	++

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)	SAR
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	++

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	+
3α,4,5,6,7,7α-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 isomers)	1335-46-2	negative	none	+
TERPINEOL ACETATE (Isomer mixture)	8007-35-0	negative	none	+
alpha-TERPINYL ACETATE	80-26-2	negative	none	+
CITRONELLYL NITRILE	51566-62-2	none	none	++

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)	SAR
alpha-CYCLOHEXYLIDENE BENZENEACETONITRILE	10461-98-0	none	none	+
DECANAL	112-31-2	none	none	++
DIHYDROMYRCENOL	18479-58-8	none	none	+
2,4-DIMETHYL-3-CYCLOHEXEN-1-CARBOXALDEHYDE	68039-49-6	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	+
Longifolene	475-20-7	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	
LITSEA CUBEBA	90063-59-5	Limited	none	
PELARGONIUM ROSEUM	90082-55-6	Limited	none	

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)	SAR
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, an exact EC3 could not be established, as the substance had been tested in too low concentration(s).
- Two single substances; 2,4-dimethyl-3-cyclohexen-1-carboxaldehyde (CAS no. 68039-49-6) and longifolene (CAS no. 475-20-7), and two natural extracts *Citrus paradisi* (CAS no. 8016-20-4) and *Mentha arvensis* (CAS no. 68917-18-0) 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, following a precautionary approach, the four substances/substance mixtures should be treated as *likely contact allergens*.
- 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. Of those, 12 chemicals and eight natural extracts are considered of special concern as they have given rise to at least 100 reported cases (listed in Table 13-5). 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)

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. Important indicative, but not exhaustive, examples include isoeugenol and its esters, geraniol and its esters, eugenol and its esters, and linalool and its esters.

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 known contact allergens. Important indicative examples include limonene, linalool, linalyl acetate, geraniol, geranial, alpha-terpinene, eugenol, isoeugenol and cinnamyl alcohol.

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 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.

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 such 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.

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, the general threshold limit (100 ppm in cosmetic products) can be applied.

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 (226). 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 (224) 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." (281) 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 µg/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 thresholds 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. 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 potent allergens 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 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 activated instead of the compound that they protect and might act themselves as skin sensitisers after oxidation. This is the case for alpha-terpinene which is described as the antioxidant in tea tree oil (Rudbäck J, Karlberg A-T et al, Chem Res Toxicol, manuscript submitted). 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	
Isoeugenol 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.

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 hypothesis must be followed by experimental investigations for the actual compounds.

Conclusions - Question 3

Many fragrance substances can act as prehaptens or prohaptens, forming potent allergens 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 are 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 to known contact allergens should be treated as equivalent to these contact allergens, i.e the same restrictions and other regulatory requirements should apply.

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
pet.	Petrolatum (as vehicle)
ppm	parts per million (1000 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

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: *Contact Dermatitis*, 4 edn, P J Frosch, T Menné and J P Lepoittevin (ed)^(eds): 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: 80-6.

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: 911-8.

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: 419-22.

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: 291-6.

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 dermato-venereologica* 1997: 77: 149-53.

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: 161-5.

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: 317-23.

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: 304-5.

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: 268-73.

17 Edman B. The influence of shaving method on perfume allergy. *Contact Dermatitis* 1994: 31: 291-2.

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: 32-6.

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: 20-1.

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: 276-9.

23 Nakayama H. Perfume allergy and cosmetic dermatitis (in Japanese). *Jpn J Dermatol* 1974: 84: 659–67.

24 Nakayama H, Harada R, Toda M. Pigmented cosmetic dermatitis. *International journal of dermatology* 1981: 15: 673–5.

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: 429-33.

30 Millqvist E, Bende M, Lowhagen O. Sensory hyperreactivity--a possible mechanism underlying cough and asthma-like symptoms. *Allergy* 1998: 53: 1208-12.

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: 1197-203.

32 Larsen W G. Perfume Dermatitis. A Study of 20 Patients. *Arch Dermatol* 1977: 113: 623-6.

33 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: 207-15.

34 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: 216-25.

35 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: 129-33.

36 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: 205-10.

37 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: 325-30.

38 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: 162-6.

39 Lunder T, Kansky A. Increase in contact allergy to fragrances: patch-test results 1989-1998. *Contact Dermatitis* 2000: 43: 107-9.

40 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.

41 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: 254-61.

42 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: 217-23.

43 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: 184-5.

44 Hendriks S A, Van Ginkel C J. Evaluation of the fragrance mix in the European standard series. *Contact Dermatitis* 1999: 41: 161-2.

45 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: 134-41.

46 Brites M M, Goncalo M, Figueiredo A. Contact allergy to fragrance mix--a 10-year study. *Contact Dermatitis* 2000: 43: 181-2.

47 Katsarma G, Gawkrodger D J. Suspected fragrance allergy requires extended patch testing to individual fragrance allergens. *Contact Dermatitis* 1999: 41: 193-7.

48 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: 220-5.

49 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: 238-44.

50 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: 136-45.

51 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.

52 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: 31-8.

53 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: 320-3.

54 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: 50-1.

55 Gupta N, Shenoi S D, Balachandran C. Fragrance sensitivity in allergic contact dermatitis. *Contact Dermatitis* 1999: 40: 53-4.

56 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: 103-7.

57 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: 73-6.

58 Kashani M N, Gorouhi F, Behnia F, Nazemi M J, Dowlati Y, Firooz A. Allergic contact dermatitis in Iran. *Contact Dermatitis* 2005: 52: 154-8.

59 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: 333-7.

60 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: 188-91.

61 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: 23-8.

62 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: 149-60.

63 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: 146-9.

64 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.

65 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: 262-9.

66 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).

67 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: 333-42.

68 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: 1076-83.

69 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.

70 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.

71 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: 295-7.

72 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.

73 Api A M. Only Peru Balsam extracts or distillates are used in perfumery. *Contact Dermatitis* 2006: 54: 179.

74 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: 304-6.

75 Cachao P, Menezes Brandao F, Carmo M, Frazao S, Silva M. Allergy to oil of turpentine in Portugal. *Contact Dermatitis* 1986: 14: 205-8.

76 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: 169-72.

77 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.

78 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: 1192-6.

79 Meding B, Swanbeck G. Occupational hand eczema in an industrial city. *Contact Dermatitis* 1990: 22: 13-23.

80 Nielsen N H, Menné T. Allergic contact sensitization in an unselected Danish population - the Glostrup allergy study, Denmark. *Acta Dermatol Venerol (Stockh)* 1992: 72: 456-60.

81 Meneghini C L, Sertoli A, Nava C, Angelini G, Francalani S, Foti C, Moroni P. Irritant contact dermatitis of the hands in housewives. In: *Irritant Dermatitis New Clinical and Experimental Aspects Curr Probl Dermatol*edn, P Elsner and H I Maibach (ed)^(eds): Basel, Karger, 1995: 41-48.

82 Seidenari S, Manzini B M, Danese P, Motolese A. Patch and prick test study of 593 healthy subjects. *Contact Dermatitis* 1990: 23: 162-7.

83 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.

84 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.

85 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 dermato-venereologica* 2001: 81: 31-4.

86 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.

87 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: 986-9.

88 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. *Occupational and environmental medicine* 2001: 58: 392-8.

89 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: 10-5.

90 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: 726-35.

91 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: 1848-53.

92 Bruze M. What is a relevant contact allergy? *Contact Dermatitis* 1990: 23: 224-5.

93 Ale I, Maibach H I. Clinical Relevance in Allergic Contact Dermatitis. An algorithmic approach. *Derm Beruf Umwelt* 1995: 43: 119-21.

94 Wahlberg J E, Lindberg M. Patch Testing. In: *Contact Dermatitis*edn, P J Frosch, T Menné and J P Lepoittevin (ed)^(eds): Berlin, Springer, 2006: 365-390.

95 Lindberg M, Matura M. Patch Testing. In: *Contact Dermatitis*, 5 edn, J D Johansen, P Frosch and J P Lepoittevin (ed)^(eds): Heidelberg etc., , Springer, 2011: 439-64.

96 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: 279-87.

97 Rothenborg H W, Hjorth N. Allergy to perfumes from toilet soaps and detergents in patients with dermatitis. *Arch Dermatol* 1968: 97: 417-21.

98 Hannuksela M, Kousa M, Pirila V. Allergy to ingredients of vehicles. *Contact Dermatitis* 1976: 2: 105-10.

99 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.

100 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.

101 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.

102 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.

103 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.

104 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.

105 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: 374-9.

106 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.

107 Skoet R, Zachariae R, Agner T. Contact dermatitis and quality of life: a structured review of the literature. *Br J Dermatol* 2003: 149: 452-6.

108 Moberg C, Alderling M, Meding B. Hand eczema and quality of life: a population-based study. *Br J Dermatol* 2009: 161: 397-403.

109 Meding B, Swanbeck G. Consequences of having hand eczema. *Contact Dermatitis* 1990: 23: 6-14.

110 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: 291-6.

111 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: 320-4.

112 Meding B, Wrangsjo K, Jarvholm B. Fifteen-year follow-up of hand eczema: predictive factors. *The Journal of investigative dermatology* 2005: 124: 893-7.

113 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: 101-8.

114 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.

115 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: 770-5.

116 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: 1020-4.

117 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: 1507-15.

118 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: 807-14.

119 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: 308-13.

120 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: 230-7.

121 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.

122 Karlberg A T, Boman A, Melin B. Animal experiments on the allergenicity of d-limonene--the citrus solvent. *Ann Occup Hyg* 1991: 35: 419-26.

123 Karlberg A T, Magnusson K, Nilsson U. Air oxidation of d-limonene (the citrus solvent) creates potent allergens. *Contact Dermatitis* 1992: 26: 332-40.

124 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.

125 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: 267-72.

126 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: 1697-705.

127 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.

128 Karlberg A T, Dooms-Gossens A. Contact allergy to oxidized d-limonene among dermatitis patients. *Contact Dermatitis* 1997: 36: 201-6.

129 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: 709-14.

130 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.

131 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: 320-8.

132 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: 274-9.

133 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.

134 Santucci B, Cristaudo A, Cannistraci C, Picardo M. Contact dermatitis to fragrances. *Contact Dermatitis* 1987: 16: 93-5.

135 Fregert S, Hjorth N. Results of Standard Patch Tests with Substances Abandoned. *Contact Dermatitis Newsletter* 1969: 5: 85-86.

136 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.

137 Sköld M, Karlberg A T, Matura M, Börje A. The fragrance chemical beta-caryophyllene-air oxidation and skin sensitization. *Food Chem Toxicol* 2006: 44: 538-45.

138 Buckley D A. Allergy to oxidized linalool in the UK. *Contact Dermatitis* 2011: 64: 240-1.

139 Berglund V. *Master Thesis University of Gothenburg*. 2011.

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: 143-50.

141 Karlberg A T, Magnusson K, Nilsson U. Influence of an anti-oxidant on the formation of allergenic compounds during auto-oxidation of d-limonene. *Ann Occup Hyg* 1994: 38: 199-207.

142 Smith C K, Hotchkiss S A. Enzymes and mechanisms of xenobiotic metabolism. In: *Allergic Contact Dermatitis Chemical and Metabolic Mechanisms*edn, W I Editor?)) (ed)^(eds), Taylor and Francis, London and New York, 2001: 45-87.

143 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.

144 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: 308-16.

145 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: 760-9.

146 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. *The Journal of investigative dermatology* 2007: 127: 1145-53.

147 Basketter D A. Skin Sensitization to Cinnamic Alcohol: The Role of Skin Metabolism. *Acta dermato-venereologica* 1992: 72: 264-5.

148 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: 189-99.

149 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.

150 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: 301-10.

151 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: 213-24.

152 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: 335-43.

153 Rastogi S C, Johansen J D. Significant exposures to isoeugenol derivatives in perfumes. *Contact Dermatitis* 2008: 58: 278-81.

154 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.

155 Basketter D A, Flyvholm M A, Menne T. Classification criteria for skin-sensitizing chemicals: a commentary. *Contact Dermatitis* 1999: 40: 175-82.

156 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: 439-44.

157 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.

158 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.

159 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.

160 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.

161 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.

162 Lepoittevin J P. Metabolism versus chemical transformation or pro- versus prehaptens? *Contact Dermatitis* 2006: 54: 73-4.

163 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.

164 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: 344-6.

165 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.

166 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: 141-4.

167 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: S211-5.

168 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.

169 Mitchell D M, Beck M H. Contact allergy to benzyl alcohol in a cutting oil reodorant. *Contact Dermatitis* 1988: 18: 301-2.

170 Mitchell J C. Contact hypersensitivity to some perfume materials. *Contact Dermatitis* 1975: 1: 196-9.

171 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.

172 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: 57-8.

173 Bernaola G, Escayol P, Fernandez E, De Corres L F. Contact dermatitis from methylionone fragrance. *Contact Dermatitis* 1989: 20: 71-2.

174 English J S, Rycroft R J. Allergic contact dermatitis from methyl heptine and methyl octine carbonates. *Contact Dermatitis* 1988: 18: 174-5.

175 Bhatia S P, Mcginty D, Letizia C S, Api A M. Fragrance material review on myrtenol. *Food Chem Toxicol* 2008: 46 Suppl 11: S237-40.

176 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: S247-50.

177 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: 171-2.

178 Mcginty D, Letizia C S, Api A M. Fragrance material review on phytol. *Food Chem Toxicol* 2010: 48 Suppl 3: S59-63.

179 Lapczynski A, Bhatia S P, Letizia C S, Api A M. Fragrance material review on rhodinol. *Food Chem Toxicol* 2008: 46 Suppl 11: S259-62.

180 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: S267-71.

181 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.

182 Surburg H, Panten J. *Common fragrance and flavor materials: preparation, properties and uses*, 5 edn. Weinheim, Wiley-VCH, 2006.

183 Trattner A, David M, Lazarov A. Occupational contact dermatitis due to essential oils. *Contact Dermatitis* 2008: 58: 282-4.

184 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: 297-9.

185 Bilsland D, Strong A. Allergic contact dermatitis from the essential oil of French marigold (Tagetes patula) in an aromatherapist. *Contact Dermatitis* 1990: 23: 55-6.

186 Cockayne S E, Gawkrodger D J. Occupational contact dermatitis in an aromatherapist. *Contact Dermatitis* 1997: 37: 306-7.

187 Boonchai W, Iamtharachai P, Sunthonpalin P. Occupational allergic contact dermatitis from essential oils in aromatherapists. *Contact Dermatitis* 2007: 56: 181-2.

188 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.

189 Selvaag E, Holm J O, Thune P. Allergic contact dermatitis in an aroma therapist with multiple sensitizations to essential oils. *Contact Dermatitis* 1995: 33: 354-5.

190 Romaguera C, Vilaplana J. Occupational contact dermatitis from ylang-ylang oil. *Contact Dermatitis* 2000: 43: 251.

191 Rudzki E, Grzywa Z. Allergy to perfume mixture. *Contact Dermatitis* 1986: 15: 115-6.

192 Rudzki E, Grzywa Z, Bruo W S. Sensitivity to 35 essential oils. *Contact Dermatitis* 1976: 2: 196-200.

193 Vilaplana J, Romaguera C. Contact dermatitis from the essential oil of tangerine in fragrance. *Contact Dermatitis* 2002: 46: 108.

194 Rudzki E, Grzywa Z. Sensitizing and irritating properties of star anise oil. *Contact Dermatitis* 1976: 2: 305-8.

195 Sugiura M, Hayakawa R, Kato Y, Sugiura K, Hashimoto R. Results of patch testing with lavender oil in Japan. *Contact Dermatitis* 2000: 43: 157-60.

196 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: 739-46.

197 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.

198 SCCP. *Memorandum on Hair Dye Substances and their Skin Sensitising Properties. Scientific Committee on Consumer Protection, adopted 19 December 2006*. 2006.

199 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: 344-52.

200 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: 1097-105.

201 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. *Toxicol Sci* 2004: 81: 332-43.

202 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: 1220-6.

203 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. *Toxicol Sci* 2009: 112: 164-74.

204 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.

205 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: 1228-33.

206 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.

207 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.

208 Fenn R S. Aroma chemical usage trends in modern perfumery. *Perfumer Flavorist* 1989: 14: 1-10.

209 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.

210 Rastogi S C, Johansen J D, Menne T. Natural ingredients based cosmetics. Content of selected fragrance sensitizers. *Contact Dermatitis* 1996: 34: 423-6.

211 Müller P M, Lamparsky D. *Perfumes: Art Science and Technology*. London, Elsevier Applied Science, 1991.

212 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.

213 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.

214 Buckley D A. Fragrance ingredient labelling in products on sale in the U.K. *Br J Dermatol* 2007: 157: 295-300.

215 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.

216 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.

217 Rastogi S C, Menne T, Johansen J D. The composition of fine fragrances is changing. *Contact Dermatitis* 2003: 48: 130-2.

218 Rastogi S C, Johansen J D, Bossi R. Selected important fragrance sensitizers in perfumes--current exposures. *Contact Dermatitis* 2007: 56: 201-4.

219 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: 367-70.

220 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: 84-8.

221 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.

222 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: 229-35.

223 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.

224 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.

225 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: 272-5.

226 Sccp. *Opinion on Hydroxyisohexyl 3-cyclohexene carboxaldehyde (sensitisation only). Scientific Committee on Consumer Products. Adopted 7 December 2004*. 2004.

227 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: 303-13.

228 Fisher A A. Cosmetic dermatitis in childhood. *Cutis* 1995: 55: 15-6.

229 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.

230 Hartmann K, Hunzelmann N. Allergic contact dermatitis from cinnamon as an odour-neutralizing agent in shoe insoles. *Contact Dermatitis* 2004: 50: 253-4.

231 Murphy L A, White I R. Contact dermatitis from geraniol in washing-up liquid. *Contact Dermatitis* 2003: 49: 52.

232 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: 109-12.

233 Topham E J, Wakelin S H. D-Limonene contact dermatitis from hand cleansers. *Contact Dermatitis* 2003: 49: 108-9.

234 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: 164-5.

235 Rastogi S C, Heydorn S, Johansen J D, Basketter D A. Fragrance chemicals in domestic and occupational products. *Contact Dermatitis* 2001: 45: 221-5.

236 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: 265-72.

237 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: 279-83.

238 Nadiminti H, Ehrlich A, Udey M C. Oral erosions as a manifestation of allergic contact sensitivity to cinnamon mints. *Contact Dermatitis* 2005: 52: 46-7.

239 Hoskyn J, Guin J D. Contact allergy to cinnamal in a patient with oral lichen planus. *Contact Dermatitis* 2005: 52: 160-1.

240 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.

241 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: 315-6.

242 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: 192-9.

243 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". *International archives of occupational and environmental health* 2004: 77: 543-51.

244 Decapite T J, Anderson B E. Allergic contact dermatitis from cinnamic aldehyde found in an industrial odour-masking agent. *Contact Dermatitis* 2004: 51: 312-3.

245 Schubert H J. Skin diseases in workers at a perfume factory. *Contact Dermatitis* 2006: 55: 81-3.

246 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: 133-9.

247 Hannuksela M. Sensitivity of Various Skin Sites in the Repeated Open Application Test. *Am J Contact Dermatitis* 1991: 2: 102-4.

248 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.

249 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.

250 Degroot A C, Frosch P J. Adverse reactions to fragrances. A clinical review. *Contact Dermatitis* 1997: 36: 57-86.

251 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: 231-7.

252 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: 117-21.

253 Uter W, Schnuch A, Gefeller O. Guidelines for the descriptive presentation and statistical analysis of contact allergy data. *Contact Dermatitis* 2004: 51: 47-56.

254 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.

255 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: 155-99.

256 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.

257 Wahlberg J E, Liden C. Cross-reactivity patterns of cobalt and nickel studied with repeated open applications (ROATS) to the skin of guinea pigs. *American journal of contact dermatitis : official journal of the American Contact Dermatitis Society* 2000: 11: 42-8.

258 Suskind R R. The hydroxycitronellal story: What can we learn from it? In: *Fragrances Beneficial and adverse effects*edn, P J Frosch, J D Johansen and I R White (ed)^(eds): Berlin, Heidelberg, New York, Springer, 1988: 159-165.

259 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.

260 SCCP. Opinion on Dermal Sensitisation Quantitative Risk Assessment (Citral, Farnesol and Phenylacetaldehyde). Scientific Committee for on Consumer Protection, adopted 24 June 2008. 2008.

261 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: 201-8.

262 Roberts D W. QSAR for upper-respiratory tract irritation. *Chem Biol Interact* 1986: 57: 325-45.

263 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.

264 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.

265 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: 337-42.

266 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: 307-12.

267 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.

268 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: 70-4.

269 Pasche F, Hunziker N. Sensitization to Kathon CG in Geneva and Switzerland. *Contact Dermatitis* 1989: 20: 115-9.

270 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: 723-9.

271 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: 1138-43.

272 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.

273 Thyssen J P, Johansen J D, Menne T, Nielsen N H, Linneberg A. Nickel allergy in Danish women before and after nickel regulation. *N Engl J Med* 2009: 360: 2259-60.

274 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: 342-8.

275 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: 83-5.

276 Braendstrup P, Johansen J D. Hydroxyisohexyl 3-cyclohexene carboxaldehyde (Lyral) is still a frequent allergen. *Contact Dermatitis* 2008: 59: 187-8.

277 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.

278 Api A M, Vey M. A new IFRA Standard on the fragrance ingredient, hydroxyisohexyl 3-cyclohexene carboxaldehyde. *Contact Dermatitis* 2010: 62: 254-5.

279 Anonymous. *OECD Guidelines for the Testing of Chemicals / Section 4: Health Effects. Test No. 429: Skin Sensitisation (Local Lymph Node Assay)*. Paris, OECD, 2002.

280 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.

281 SCCP. *Opinion on Oak moss/Tree moss (sensitisation only) Scientific Committee on Consumer Products, adopted 15 April 2008*. 2008.

MATERIAL SAFETY DATA SHEET

Cedarwood Oil (Virginia)

Prepared to U.S. OSHA, CMA, ANSI, Canadian WHMIS, Australian WorkSafe, Japanese Industrial Standard JIS Z 7250:2000, and European Union REACH Regulations

SECTION 1 - PRODUCT AND COMPANY IDENTIFICATION

PRODUCT NAME:	**Cedarwood Oil (Virginia)**
CAS NUMBER:	85085-41-2
U.N. NUMBER:	Not Applicable
U.N. DANGEROUS GOODS CLASS:	Non-Regulated Material
MANUFACTURER'S NAME:	**Grayden Cedarworks, Inc.**
ADDRESS:	8782 Ranch Road 2169 East, Junction, TX 76849 USA
EMERGENCY PHONE:	325-446-3366
BUSINESS PHONE:	325-446-3366
FAX:	325-446-3367
DATE OF PREPARATION:	June 12, 2012
DATE OF LAST REVISION:	New

SECTION 2 - HAZARDS IDENTIFICATION

EMERGENCY OVERVIEW: This product is a pale yellow liquid with a cedar wood odor. Prolonged contact with skin may cause irritation. Contact with eyes may cause irritation. Product will support combustion above flashpoint. This product has not been investigated to determine adverse environmental effects.

US DOT SYMBOLS	CANADA (WHMIS) SYMBOLS	EUROPEAN and (GHS) Hazard Symbols
Non-Regulated	Not Controlled	

Signal Word: **Warning!**

EU LABELING AND CLASSIFICATION:

Classification of the substance or mixture according to Regulation (EC) No1272/2008 Annex 1
EC# 285-370-3 This substance is not classified in the Annex I of Directive 67/548/EEC

GHS Hazard Classification(s):
None

Hazard Statement(s):
H316: Causes mild skin irritation
H320: Causes eye irritation

Precautionary Statement(s):
P264: Wash hands thoroughly after handling
P280: Wear protective gloves/protective clothing/eye protection/face protection

Hazard Symbol(s):
[Xi] Irritant

Risk Phrases:
R36/38: Irritating to eyes and skin

Safety Phrases:
S24/25: Avoid contact with skin and eyes.
S36/37: Wear suitable protective clothing and gloves.

HEALTH HAZARDS OR RISKS FROM EXPOSURE:

ACUTE: Exposure to this product may cause irritation of the eyes with redness and pain. Prolonged contact with skin may cause irritation with redness. Inhalation of this product may cause irritation to the respiratory tract. Ingestion may cause gastrointestinal irritation including vomiting or diarrhea.

CHRONIC: None known

TARGET ORGANS: ACUTE: Eyes, Skin, Respiratory System CHRONIC: None Known

SECTION 3 - COMPOSITION and INFORMATION ON INGREDIENTS

HAZARDOUS INGREDIENTS:	CAS #	EINECS #	ICSC #	WT %	HAZARD CLASSIFICATION; RISK PHRASES
Cedarwood Oil (Juniper, Juniperus virginiana, ext)	85085-41-2	285-370-3	Not Listed	100%	HAZARD CLASSIFICATION: [Xi] Irritant RISK PHRASES: R36/38
Balance of other ingredients are non-hazardous or less than 1% in concentration (or 0.1% for carcinogens, reproductive toxins, or respiratory sensitizers).					

NOTE: ALL WHMIS required information is included in appropriate sections based on the ANSI Z400.1-2010 format. This product has been classified in accordance with the hazard criteria of the CPR and the MSDS contains all the information required by the CPR, EU Directives and the Japanese Industrial Standard *JIS Z 7250: 2000*.

SECTION 4 - FIRST-AID MEASURES

Contaminated individuals of chemical exposure must be taken for medical attention if any adverse effect occurs. Rescuers should be taken for medical attention, if necessary. Take copy of label and MSDS to health professional with contaminated individual.

EYE CONTACT: If product enters the eyes, open eyes while under gentle running water for at least 15 minutes. Seek medical attention if irritation persists.

SKIN CONTACT: Wash skin thoroughly after handling. Seek medical attention if irritation develops and persists. Remove contaminated clothing. Launder before re-use.

INHALATION: If breathing becomes difficult, remove victim to fresh air. If necessary, use artificial respiration to support vital functions. Seek medical attention if breathing dificulty continues.

INGESTION: If product is swallowed, call physician or poison control center for most current information. If professional advice is not available, do not induce vomiting. Never induce vomiting or give diluents (milk or water) to someone who is unconscious, having convulsions, or who cannot swallow. Seek medical advice. Take a copy of the label and/or MSDS with the victim to the health professional.

MEDICAL CONDITIONS AGGRAVATED BY EXPOSURE: None known

RECOMMENDATIONS TO PHYSICIANS: Treat symptoms and reduce over-exposure.

SECTION 5 - FIRE-FIGHTING MEASURES

FLASH POINT: >110 deg C (>230.00 deg F)

AUTOIGNITION TEMPERATURE: Not Established

FLAMMABLE LIMITS (in air by volume, %): Lower (LEL): Not Available Upper (UEL): Not Available

FIRE EXTINGUISHING MATERIALS: Carbon dioxide, foam, dry chemical, halon, or water fog.

UNUSUAL FIRE AND EXPLOSION HAZARDS: This product will support combustion above flashpoint. This liquid floats on water and may travel to a source of ignition and spread fire.

Explosion Sensitivity to Mechanical Impact: None

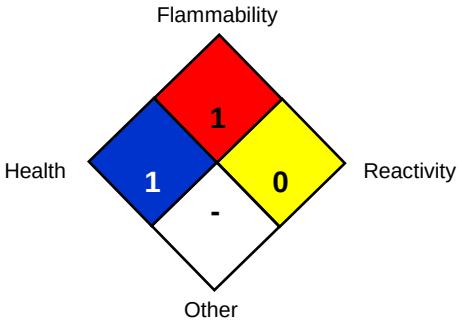
Explosion Sensitivity to Static Discharge: None

SPECIAL FIRE-FIGHTING PROCEDURES: Incipient fire responders should wear eye protection. Structural firefighters must wear Self-Contained Breathing Apparatus and full protective equipment. Isolate materials not yet involved in the fire and protect personnel. Move containers from fire area if this can be done without risk; otherwise, cool with carefully applied water spray. If possible, prevent runoff water from entering storm drains, bodies of water, or other environmentally sensitive areas.

MATERIAL SAFETY DATA SHEET

Cedarwood Oil (Virginia)

NFPA RATING SYSTEM



HMIS RATING SYSTEM

HAZARDOUS MATERIAL IDENTIFICATION SYSTEM

HEALTH HAZARD (BLUE)	1
FLAMMABILITY HAZARD (RED)	1
PHYSICAL HAZARD (YELLOW)	0

PROTECTIVE EQUIPMENT			
EYES	RESPIRATORY	HANDS	BODY
	See Sect 8		See Sect 8

For Routine Industrial Use and Handling Applications

Hazard Scale: **0** = Minimal **1** = Slight **2** = Moderate **3** = Serious **4** = Severe * = Chronic hazard

SECTION 6 - ACCIDENTAL RELEASE MEASURES

SPILL AND LEAK RESPONSE: Personnel should be trained for spill response operations.
SPILLS: Contain spill if safe to do so. Prevent entry into drains, sewers, and other waterways. Soak up with an absorbent material and place in an appropriate container for disposal. Dispose of in accordance with applicable Federal, State, and local procedures (see Section 13, Disposal Considerations).

SECTION 7 - HANDLING and STORAGE

WORK PRACTICES AND HYGIENE PRACTICES: As with all chemicals, avoid getting this product ON YOU or IN YOU. Wash thoroughly after handling this product. Do not eat, drink, smoke, or apply cosmetics while handling this product. Avoid breathing vapors.mists/sprays generated by this product. Use in a well-ventilated location. Remove contaminated clothing immediately.

STORAGE AND HANDLING PRACTICES: Containers of this product must be properly labeled. Store containers in a cool, dry location. Keep container tightly closed when not in use. Store away from incompatible materials.

SECTION 8 - EXPOSURE CONTROLS - PERSONAL PROTECTION

EXPOSURE LIMITS/GUIDELINES:

Chemical Name	CAS#	ACGIH TWA	OSHA TWA	SWA
Cedarwood Oil (Juniper, Juniperus virginiana, ext)	85085-41-2	Not Listed	Not Listed	Not Listed

Currently, International exposure limits are not established for the components of this product. Please check with competent authority in each country for the most recent limits in place.

VENTILATION AND ENGINEERING CONTROLS: Use with adequate ventilation to ensure exposure levels are maintained below the limits provided below. Use local exhaust ventilation to control airborne mist/vapors. Ensure eyewash/safety shower stations are available near areas where this product is used.

The following information on appropriate Personal Protective Equipment is provided to assist employers in complying with OSHA regulations found in 29 CFR Subpart I (beginning at 1910.132) or equivalent standard of Canada, or standards of EU member states (including EN 149 for respiratory PPE, and EN 166 for face/eye protection), and those of Japan. Please reference applicable regulations and standards for relevant details.

RESPIRATORY PROTECTION: Maintain airborne contaminant concentrations below guidelines listed above, if applicable. If necessary, use only respiratory protection authorized in the U.S. Federal OSHA Respiratory Protection Standard (29 CFR 1910.134), equivalent U.S. State standards, Canadian CSA Standard Z94.4-93, the European Standard EN149, or EU member states.

EYE PROTECTION: Safety glasses as appropriate to avoid eye contact. If necessary, refer to U.S. OSHA 29 CFR 1910.133 or appropriate Canadian Standards.

HAND PROTECTION: Use chemical resistant gloves to prevent skin contact. If necessary, refer to U.S. OSHA 29 CFR 1910.138 or appropriate Standards of Canada.

BODY PROTECTION: Use body protection appropriate to prevent contact (e.g. lab coat, overalls). If necessary, refer to appropriate Standards of Canada, or appropriate Standards of the EU, Australian Standards, or relevant Japanese Standards.

SECTION 9 - PHYSICAL and CHEMICAL PROPERTIES

PHYSICAL STATE:	Liquid
APPEARANCE & ODOR:	Pale yellow with a cedar wood odor
ODOR THRESHOLD (PPM):	Not Available
VAPOR PRESSURE (mmHg):	0.017100 mm/Hg @ 25.00 °C. (est)
VAPOR DENSITY (AIR=1):	>1
BY WEIGHT:	Not Available
EVAPORATION RATE (nBuAc = 1):	<1
BOILING POINT (C°):	279°C. @ 760.00 mm Hg
FREEZING POINT (C°):	Not Available.
pH:	Not Applicable
SPECIFIC GRAVITY 20°C: (WATER =1)	0.95
SOLUBILITY IN WATER (%)	Negligible
COEFFICIENT OF WATER/OIL DIST.:	Not Available

SECTION 10 - STABILITY and REACTIVITY

STABILITY: Product is stable

DECOMPOSITION PRODUCTS: When heated to decomposition this product produces Oxides of carbon and (COx),Hydrocarbons.

MATERIALS WITH WHICH SUBSTANCE IS INCOMPATIBLE: Strong oxidizing agents.

HAZARDOUS POLYMERIZATION: Will not occur.

CONDITIONS TO AVOID: Incompatible materials.

SECTION 11 - TOXICOLOGICAL INFORMATION

TOXICITY DATA: CAS# 85085-41-2

Oral, rat: LD50	>5 gm/kg;	
Draize test, rabbit, skin:	500 mg/24H	Moderate;
Skin, rabbit: LD50	>5 gm/kg;	

SUSPECTED CANCER AGENT: None of the ingredients are found on the following lists: FEDERAL OSHA Z LIST, NTP, CAL/OSHA, IARC and therefore is not considered to be, nor suspected to be a cancer-causing agent by these agencies.

IRRITANCY OF PRODUCT: Contact with this product can be irritating to exposed skin and eyes.

SENSITIZATION OF PRODUCT: This product is not considered a sensitizer.

REPRODUCTIVE TOXICITY INFORMATION: No information concerning the effects of this product and its components on the human reproductive system.

SECTION 12 - ECOLOGICAL INFORMATION

ALL WORK PRACTICES MUST BE AIMED AT ELIMINATING ENVIRONMENTAL CONTAMINATION.

ENVIRONMENTAL STABILITY: No Data available at this time.

EFFECT OF MATERIAL ON PLANTS or ANIMALS: This product has not been investigated as to the effects on plants or animals.

EFFECT OF CHEMICAL ON AQUATIC LIFE: This product has not been investigated as to the effects on aquatic life.

SECTION 13 - DISPOSAL CONSIDERATIONS

PREPARING WASTES FOR DISPOSAL: Waste disposal must be in accordance with appropriate Federal, State, and local regulations, those of Canada, Australia, EU Member States and Japan.

SECTION 14 - TRANSPORTATION INFORMATION

U.S. DOT; IATA; IMO; ADR:

THIS PRODUCT IS NOT HAZARDOUS AS DEFINED BY 49 CFR 172.101 BY THE U.S. DEPARTMENT OF TRANSPORTATION.

PROPER SHIPPING NAME: Non-Regulated Material

HAZARD CLASS NUMBER and DESCRIPTION: Not Applicable

UN IDENTIFICATION NUMBER: Not Applicable

PACKING GROUP: Not Applicable

DOT LABEL(S) REQUIRED: Not Applicable

NORTH AMERICAN EMERGENCY RESPONSE GUIDEBOOK NUMBER (2004): Not Applicable

MARINE POLLUTANT: None of the ingredients are classified by the DOT as a Marine Pollutant (as defined by 49 CFR 172.101, Appendix B)

U.S. DEPARTMENT OF TRANSPORTATION (DOT) SHIPPING REGULATIONS:

This product is not classified as dangerous goods, per U.S. DOT regulations, under 49 CFR 172.101.

TRANSPORT CANADA, TRANSPORTATION OF DANGEROUS GOODS REGULATIONS:

This product is not classified as Dangerous Goods, per regulations of Transport Canada.

INTERNATIONAL AIR TRANSPORT ASSOCIATION (IATA):

This product is not classified as Dangerous Goods, by rules of IATA:

INTERNATIONAL MARITIME ORGANIZATION (IMO) DESIGNATION:

This product is not classified as Dangerous Goods by the International Maritime Organization.

EUROPEAN AGREEMENT CONCERNING THE INTERNATIONAL CARRIAGE OF DANGEROUS GOODS BY ROAD (ADR):

This product is not classified by the United Nations Economic Commission for Europe to be dangerous goods.

SECTION 15 - REGULATORY INFORMATION

UNITED STATES REGULATIONS

SARA REPORTING REQUIREMENTS: This product is not subject to the reporting requirements of Sections 302, 304 and 313 of Title III of the Superfund Amendments and Reauthorization Act., as follows: None

TSCA: All components in this product are listed on the US Toxic Substances Control Act (TSCA) inventory of chemicals.

SARA 311/312:

Acute Health:	Yes	Chronic Health:	No	Fire:	Yes	Reactivity:	No

U.S. SARA THRESHOLD PLANNING QUANTITY: There are no specific Threshold Planning Quantities for this product. The default Federal MSDS submission and inventory requirement filing threshold of 10,000 lb (4,540 kg) may apply, per 40 CFR 370.20.

U.S. CERCLA REPORTABLE QUANTITY (RQ): None

CALIFORNIA SAFE DRINKING WATER AND TOXIC ENFORCEMENT ACT (PROPOSITION 65): None of the ingredients are on the California Proposition 65 lists.

CANADIAN REGULATIONS:

CANADIAN DSL/NDSL INVENTORY STATUS: All of the components of this product are on the DSL Inventory

CANADIAN ENVIRONMENTAL PROTECTION ACT (CEPA) PRIORITIES SUBSTANCES LISTS: No component of this product is on the CEPA First Priorities Substance Lists.

CANADIAN WHMIS CLASSIFICATION and SYMBOLS: This product is not controlled

EUROPEAN ECONOMIC COMMUNITY INFORMATION:

EU LABELING AND CLASSIFICATION:

Classification of the mixture according to Regulation (EC) No1272/2008. See section 2 for details.

AUSTRALIAN INFORMATION FOR PRODUCT:

AUSTRALIAN INVENTORY OF CHEMICAL SUBSTANCES (AICS) STATUS: All components of this product are listed on the AICS.

STANDARD FOR THE UNIFORM SCHEDULING OF DRUGS AND POISONS: Not applicable.

JAPANESE INFORMATION FOR PRODUCT:

JAPANESE MINISTER OF INTERNATIONAL TRADE AND INDUSTRY (MITI) STATUS: The components of this product are not listed as Class I Specified Chemical Substances, Class II Specified Chemical Substances, or Designated Chemical Substances by the Japanese MITI.

INTERNATIONAL CHEMICAL INVENTORIES:

Listing of the components on individual country Chemical Inventories is as follows:

Asia-Pac:	Listed
Australian Inventory of Chemical Substances (AICS):	Listed
Korean Existing Chemicals List (ECL):	Listed
Japanese Existing National Inventory of Chemical Substances (ENCS):	Listed
Philippines Inventory if Chemicals and Chemical Substances (PICCS):	Listed
Swiss Giftliste List of Toxic Substances:	Listed
U.S. TSCA:	Listed

SECTION 16 - OTHER INFORMATION

PREPARED BY: Paul Eigbrett

MSDS Authoring PLUS
www.msdsauthoringplus.com

Disclaimer: To the best knowledge of Grayden Cedarworks, Inc., the information contained herein is reliable and accurate as of this date; however, accuracy, suitability or completeness is not guaranteed and no warranties of any type either express or implied are provided. The information contained herein relates only to this specific product.

SAFETY DATA SHEET

Creation Date 11-Jan-2011 **Revision Date** 26-May-2017 **Revision Number** 2

1. Identification

Product Name **Cedarwood oil**

Cat No. : **AC612080000; AC612085000**

Synonyms red cedarwood oil.; Oil cedar

Recommended Use Laboratory chemicals.
Uses advised against Not for food, drug, pesticide or biocidal product use

Details of the supplier of the safety data sheet

Company
Fisher Scientific
One Reagent Lane
Fair Lawn, NJ 07410
Tel: (201) 796-7100

Acros Organics
One Reagent Lane
Fair Lawn, NJ 07410

Emergency Telephone Number
For information **US** call: 001-800-ACROS-01 / **Europe** call: +32 14 57 52 11
Emergency Number **US:**001-201-796-7100 / **Europe:** +32 14 57 52 99
CHEMTREC Tel. No.**US:**001-800-424-9300 / **Europe:**001-703-527-3887

2. Hazard(s) identification

Classification
Classification under 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200)

Based on available data, the classification criteria are not met

Label Elements
None required

Hazards not otherwise classified (HNOC)
None identified

3. Composition / information on ingredients

Component	CAS-No	Weight %
Cedar wood oil	8000-27-9	100

4. First-aid measures

Eye Contact Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Get medical attention if symptoms occur.

Skin Contact Wash off immediately with plenty of water for at least 15 minutes. Get medical attention if symptoms occur.

Inhalation Move to fresh air. If breathing is difficult, give oxygen. Get medical attention if symptoms occur.

Ingestion Do not induce vomiting. Get medical attention if symptoms occur.

Most important symptoms/effects No information available.
Notes to Physician Treat symptomatically

5. Fire-fighting measures

Suitable Extinguishing Media CO_2, dry chemical, dry sand, alcohol-resistant foam.

Unsuitable Extinguishing Media Water may be ineffective

Flash Point 110 °C / 230 °F

Method - No information available

Autoignition Temperature No information available
Explosion Limits
Upper No data available
Lower No data available
Sensitivity to Mechanical Impact No information available
Sensitivity to Static Discharge No information available

Specific Hazards Arising from the Chemical
Thermal decomposition can lead to release of irritating gases and vapors. Keep product and empty container away from heat and sources of ignition.

Hazardous Combustion Products
Carbon monoxide (CO) Carbon dioxide (CO_2)
Protective Equipment and Precautions for Firefighters
As in any fire, wear self-contained breathing apparatus pressure-demand, MSHA/NIOSH (approved or equivalent) and full protective gear.

NFPA

Health	Flammability	Instability	Physical hazards
1	1	0	N/A

6. Accidental release measures

Personal Precautions Use personal protective equipment. Ensure adequate ventilation. Avoid contact with skin, eyes and clothing.
Environmental Precautions Avoid release to the environment.

Methods for Containment and Clean Up Soak up with inert absorbent material. Keep in suitable, closed containers for disposal.

7. Handling and storage

Handling Wear personal protective equipment. Ensure adequate ventilation. Avoid contact with skin, eyes and clothing. Avoid ingestion and inhalation.

Storage Keep containers tightly closed in a dry, cool and well-ventilated place.

8. Exposure controls / personal protection

Exposure Guidelines This product does not contain any hazardous materials with occupational exposure

limitsestablished by the region specific regulatory bodies.

Engineering Measures None under normal use conditions.

Personal Protective Equipment

Eye/face Protection Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.

Skin and body protection Wear appropriate protective gloves and clothing to prevent skin exposure.

Respiratory Protection No protective equipment is needed under normal use conditions.

Hygiene Measures Handle in accordance with good industrial hygiene and safety practice.

9. Physical and chemical properties

Physical State	Liquid
Appearance	Light yellow
Odor	Strong
Odor Threshold	No information available
pH	No information available
Melting Point/Range	No data available
Boiling Point/Range	279 °C / 534.2 °F
Flash Point	110 °C / 230 °F
Evaporation Rate	No information available
Flammability (solid,gas)	Not applicable
Flammability or explosive limits	
Upper	No data available
Lower	No data available
Vapor Pressure	No information available
Vapor Density	No information available
Specific Gravity	0.95
Solubility	Insoluble in water
Partition coefficient; n-octanol/water	No data available
Autoignition Temperature	No information available
Decomposition Temperature	No information available
Viscosity	No information available

10. Stability and reactivity

Reactive Hazard No

Stability Stable under normal conditions.

Conditions to Avoid Incompatible products. Excess heat.

Incompatible Materials Strong oxidizing agents

Hazardous Decomposition Products Carbon monoxide (CO), Carbon dioxide (CO_2)

Hazardous Polymerization Hazardous polymerization does not occur.

Hazardous Reactions None under normal processing.

11. Toxicological information

Acute Toxicity

Product Information

Oral LD50 Based on ATE data, the classification criteria are not met. ATE > 2000 mg/kg.

Dermal LD50 Based on ATE data, the classification criteria are not met. ATE > 2000 mg/kg.

Vapor LC50 Based on ATE data, the classification criteria are not met. ATE > 20 mg/l.

Component Information

Component	LD50 Oral	LD50 Dermal	LC50 Inhalation
Cedar wood oil	LD50 > 5 g/kg (Rat)	LD50 > 5 g/kg (Rabbit)	Not listed

Toxicologically Synergistic Products No information available

Delayed and immediate effects as well as chronic effects from short and long-term exposure

Irritation No information available

Sensitization No information available

Carcinogenicity The table below indicates whether each agency has listed any ingredient as a carcinogen.

Component	CAS-No	IARC	NTP	ACGIH	OSHA	Mexico
Cedar wood oil	8000-27-9	Not listed	Not listed	Not listed	Not listed	Not listed

Mutagenic Effects No information available

Reproductive Effects No information available.

Developmental Effects No information available.

Teratogenicity No information available.

STOT - single exposure None known

STOT - repeated exposure None known

Aspiration hazard No information available

Symptoms / effects,both acute and delayed No information available

Endocrine Disruptor Information No information available

Other Adverse Effects The toxicological properties have not been fully investigated.

12. Ecological information

Ecotoxicity

Do not empty into drains.

Persistence and Degradability Insoluble in water

Bioaccumulation/ Accumulation No information available.

Mobility Is not likely mobile in the environment due its low water solubility.

13. Disposal considerations

Waste Disposal Methods Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. Chemical waste generators must also consult local, regional, and national hazardous waste regulations to ensure complete and accurate classification.

14. Transport information

DOT Not regulated

TDG Not regulated
IATA Not regulated
IMDG/IMO Not regulated

15. Regulatory information

All of the components in the product are on the following Inventory lists: X = listed

International Inventories

Component	TSCA	DSL	NDSL	EINECS	ELINCS	NLP	PICCS	ENCS	AICS	IECSC	KECL
Cedar wood oil	X	X	-	-	-		X	-	X	X	X

Legend:
X - Listed
E - Indicates a substance that is the subject of a Section 5(e) Consent order under TSCA.
F - Indicates a substance that is the subject of a Section 5(f) Rule under TSCA.
N - Indicates a polymeric substance containing no free-radical initiator in its inventory name but is considered to cover the designated polymer made with any free-radical initiator regardless of the amount used.
P - Indicates a commenced PMN substance
R - Indicates a substance that is the subject of a Section 6 risk management rule under TSCA.
S - Indicates a substance that is identified in a proposed or final Significant New Use Rule
T - Indicates a substance that is the subject of a Section 4 test rule under TSCA.
XU - Indicates a substance exempt from reporting under the Inventory Update Rule, i.e. Partial Updating of the TSCA Inventory Data Base Production and Site Reports (40 CFR 710(B).
Y1 - Indicates an exempt polymer that has a number-average molecular weight of 1,000 or greater.
Y2 - Indicates an exempt polymer that is a polyester and is made only from reactants included in a specified list of low concern reactants that comprises one of the eligibility criteria for the exemption rule.

U.S. Federal Regulations

TSCA 12(b) Not applicable

SARA 313 Not applicable

SARA 311/312 Hazard Categories

Acute Health Hazard	No
Chronic Health Hazard	No
Fire Hazard	No
Sudden Release of Pressure Hazard	No
Reactive Hazard	No

CWA (Clean Water Act) Not applicable

Clean Air Act Not applicable

OSHA Occupational Safety and Health Administration
Not applicable

CERCLA
Not applicable

California Proposition 65 This product does not contain any Proposition 65 chemicals

U.S. State Right-to-Know Regulations Not applicable

U.S. Department of Transportation

Reportable Quantity (RQ):	N
DOT Marine Pollutant	N
DOT Severe Marine Pollutant	N

U.S. Department of Homeland Security

This product does not contain any DHS chemicals.

Other International Regulations

Mexico - Grade Slight risk, Grade 1

16. Other information

Prepared By
Regulatory Affairs
Thermo Fisher Scientific
Email: EMSDS.RA@thermofisher.com

Creation Date 11-Jan-2011
Revision Date 26-May-2017
Print Date 26-May-2017
Revision Summary This document has been updated to comply with the US OSHA HazCom 2012 Standard replacing the current legislation under 29 CFR 1910.1200 to align with the Globally Harmonized System of Classification and Labeling of Chemicals (GHS).

Disclaimer

The information provided in this Safety Data Sheet is correct to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guidance for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other materials or in any process, unless specified in the text

End of SDS